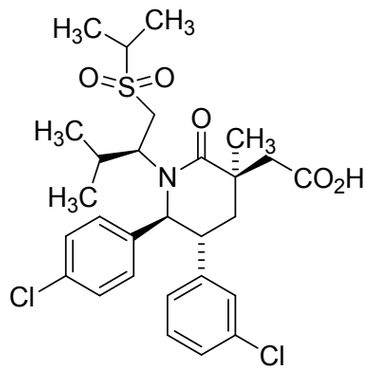
## REIVINDICACIONES

1. Un compuesto, en donde el compuesto es:




caracterizado porque el patrón de difracción de rayos-X en polvo comprende picos en los grados del ángulo de difracción 2-teta en 11.6, 12.4, 18.6, 19.0, 21.6 y 23.6.

2. El compuesto de la reivindicación 1, en donde el compuesto se caracteriza porque tiene un punto de fusión en 161°C.

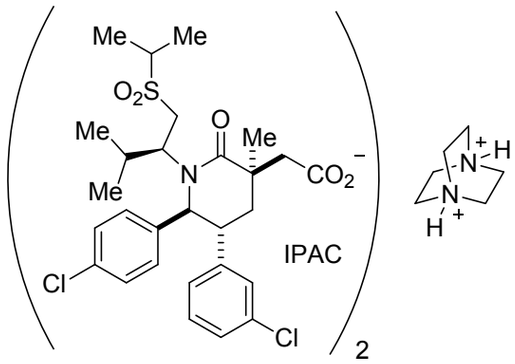
3. Una composición farmacéutica que comprende el compuesto de la reivindicación 1 y un excipiente farmacéuticamente aceptable.

4. La composición de la reivindicación 3, en donde la composición farmacéutica está en forma de una forma de dosificación sólida.

5. La composición farmacéutica de la reivindicación 4, en donde la forma de dosificación sólida es una cápsula, una tableta, polvo o gránulos.

6. La composición farmacéutica de la reivindicación 5, en donde la forma de dosificación sólida es una tableta.

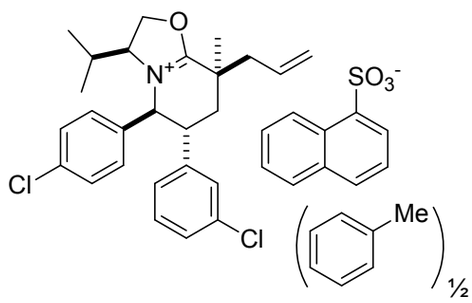
7. Un compuesto, en donde el compuesto es:




8. El compuesto de la reivindicación 7, en donde el compuesto se caracteriza porque el patrón de difracción de rayos-X en polvo comprende picos en los grados del ángulo de difracción 2-teta, en 11.5, 14.3, 15.8, 17.7, 19.5 y 20.7.

9. El compuesto de las reivindicaciones 7 u 8, en donde el compuesto se caracteriza por presentar un punto de fusión en 96°C.

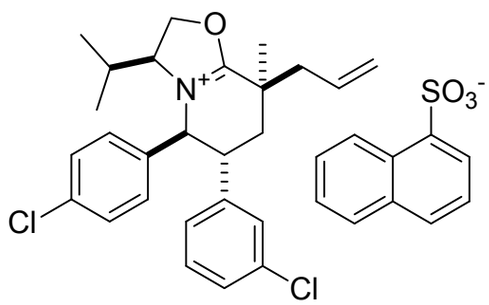
10. Un compuesto, en donde el compuesto es:



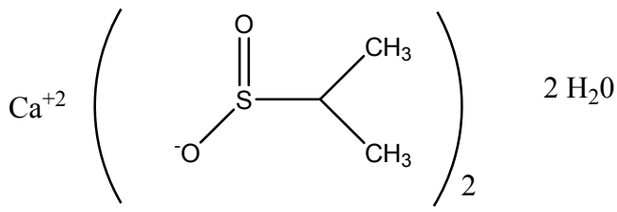

11. El compuesto de la reivindicación 10, en donde el compuesto se caracteriza porque el patrón de difracción de rayos-X en polvo comprende picos en los grados del ángulo de difracción 2-teta en 8.7, 18.5, 22.6 y 26.6.

12. El compuesto de las reivindicaciones 10 u 11, en donde el compuesto se caracteriza por presentar un punto de fusión en 208°C.

13. Un compuesto, en donde el compuesto es:



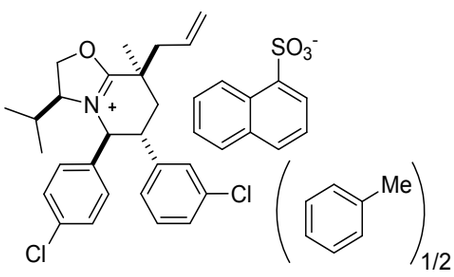

14. Un compuesto, en donde el compuesto es:



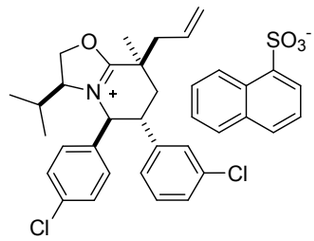
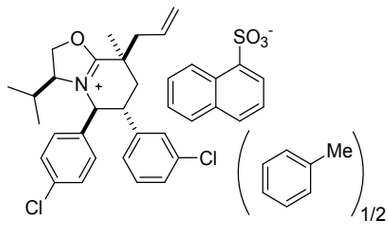

15. Un proceso para producir el compuesto de la reivindicación 14, en donde el proceso comprende hacer reaccionar ácido propano-2-silfínico con sal de calcio.

16. El proceso de la reivindicación 15, en donde la sal de calcio es sulfato de calcio.

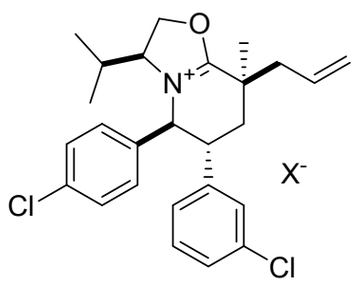
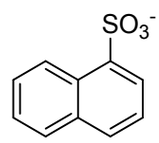
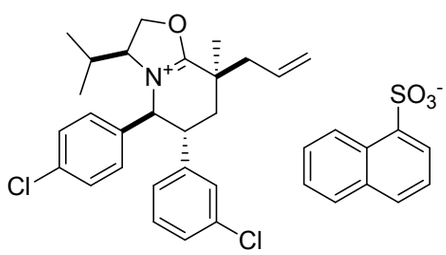
17. Un proceso para producir:

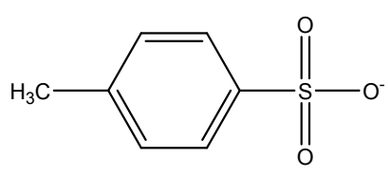


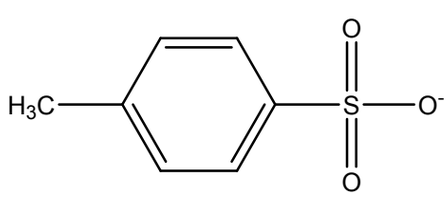

, en donde el proceso comprende:

hacer reaccionar  , con tolueno para formar  .

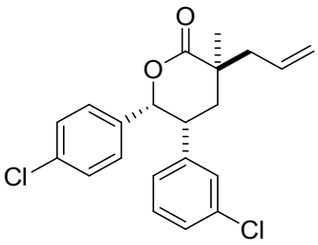
18. El proceso de la reivindicación 17, en donde el proceso además comprende hacer reaccionar:

 con  , para formar  ,

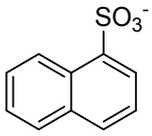
en donde $X^-$ es $CF_3SO_3^-$, o  .

19. El proceso de la reivindicación 18, en donde X es 

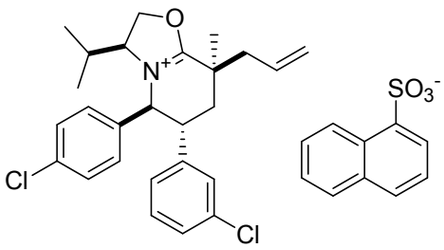
20. El proceso de la reivindicación 17, en donde el proceso además comprende hacer reaccionar:




con L-valinol, seguido de condiciones deshidratantes, y en seguida con




, para formar:

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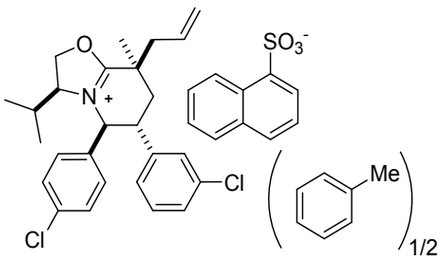
21. El proceso de la reivindicación 20, en donde las condiciones deshidratantes comprenden hacer reaccionar con anhídrido tríflico o anhídrido del ácido p-toluenosulfónico, en presencia de una base.

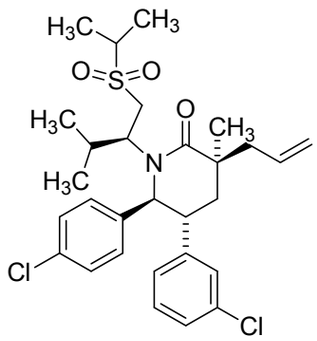
22. El proceso de la reivindicación 21, en donde la base es 2,6-lutidino.

23. El proceso de cualquiera de las reivindicaciones 17 a 22, en donde:

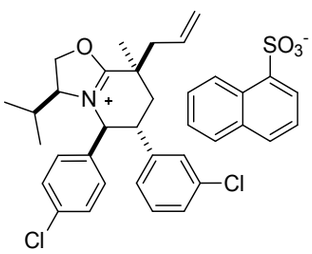



es el compuesto de las reivindicaciones 11 o 12.

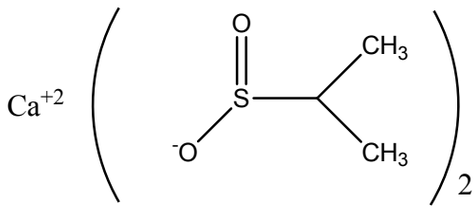
24. Un proceso para producir



en donde el proceso comprende hacer reaccionar:

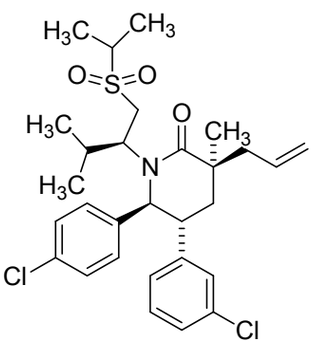


con



,

para formar:



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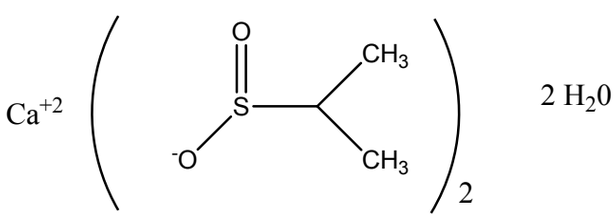
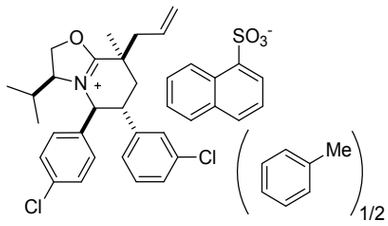
25. El proceso de la reivindicación 24, en donde el proceso además comprende

disolver

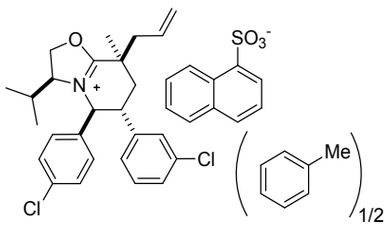


, en un solvente apropiado.

26. El proceso de la reivindicación 24, en donde el proceso además comprende

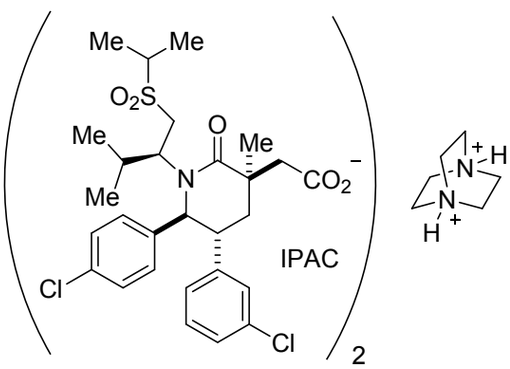
disolver  , en un solvente apropiado.

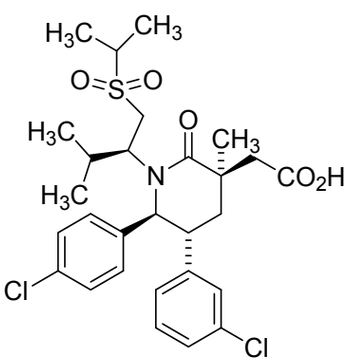
27. El proceso de la reivindicación 26, en donde:

 , es el compuesto de las reivindicaciones 11 o 12.

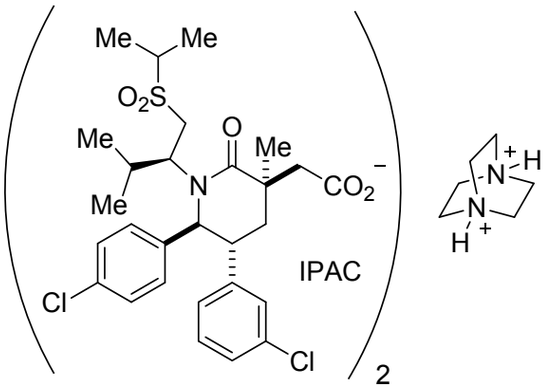
28. El proceso de cualquiera de las reivindicaciones 25 a 27, en donde el solvente apropiado es tolueno.

29. Un proceso para producir:

 , en donde el proceso comprende hacer reaccionar:



con 1,4-diazabiciclo[2.2.2]octano (DABCO), para formar:

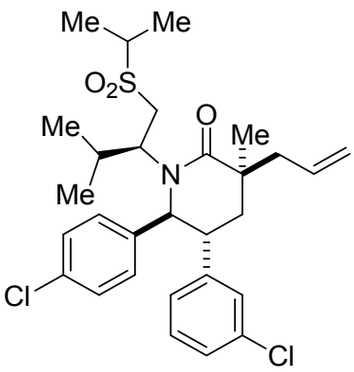




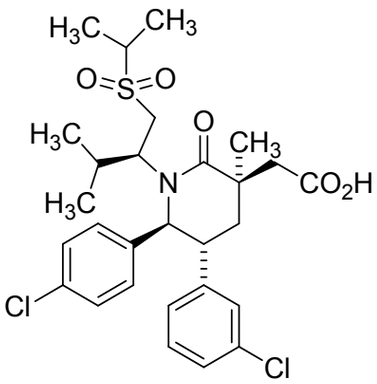


.

30. El proceso de la reivindicación 29, en donde el proceso además comprende hacer reaccionar:




, bajo condiciones de oxidación, para formar:

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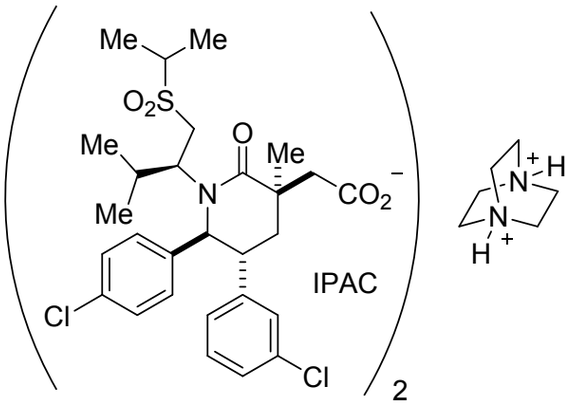
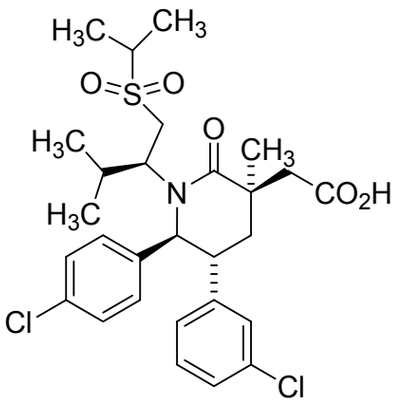
31. El proceso de la reivindicación 30, en donde las condiciones de oxidación comprenden una reacción con ozono, seguido de una oxidación Pinnick.

32. El proceso de cualquiera de las reivindicaciones 29 a 31, en donde:

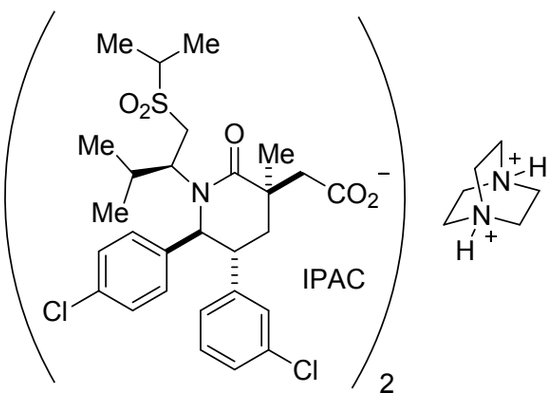



es un compuesto de las reivindicaciones 8 o 9.

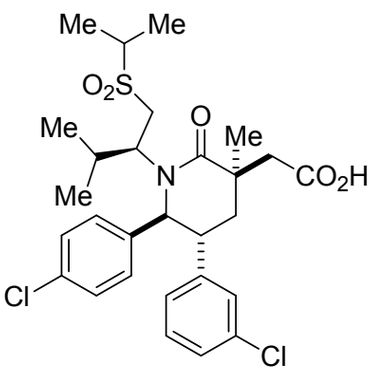
33. Un proceso para producir:




, en donde el proceso comprende hacer reaccionar:




, con un ácido para formar:

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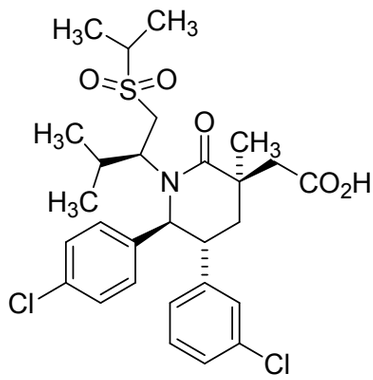
34. El proceso de la reivindicación 33, en donde el ácido es ácido clorhídrico.

35. El proceso de la reivindicación 33 o 34, en donde:




es un compuesto de las reivindicaciones 1 o 2.

**P-10921**

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
**DIRECCIÓN DE NUEVAS CREACIONES**
E. S. D.

Re: Expediente No. **15-293.617**
Solicitud de patente a nombre de **AMGEN INC.**

Yo, CAROLINA DAZA MONTALVO identificada como aparece al pie de mi firma, domiciliada en la Carrera 11 No. 86-53, piso 6, obrando como apoderada de la sociedad AMGEN INC., domiciliada en Thousand Oaks, California, Estados Unidos de América, solicitante de la patente de la referencia, en respuesta al oficio No. **8309** notificado el 12 de junio de 2017, relacionado con concepto técnico de fondo, atentamente me permito manifestar:

**1. Modificación a las Reivindicaciones**

Con fundamento en la facultad que confiere el artículo 34 de la Decisión 486 al solicitante para modificar la solicitud de patente en cualquier momento del trámite, presento junto con este memorial nuevo capítulo reivindicatorio, el cual consta de 35 reivindicaciones y reemplaza completamente el capítulo presentado anteriormente. En el nuevo capítulo reivindicatorio se realizaron las siguientes modificaciones:

**1.1** Se modificó el contenido de las reivindicaciones 1, 19 (nueva reivindicación 8) y 24 (nueva reivindicación 11), a fin de caracterizar el compuesto por sus respectivos picos, según lo solicitado por el examinador.

**1.2** No se incluyen las reivindicaciones 2, 3, 5 a 8, 13 a 17, 20, 21, 25 y 27 a 30. Las reivindicaciones restantes, así como sus dependencias han sido reenumeradas.

**1.3** Se modificó el contenido a lo largo de las reivindicaciones en vista del nuevo alcance de la reivindicación 1.

En cuanto a estas modificaciones, me permito decir que las mismas no amplían de ninguna manera la materia objeto de la presente solicitud y se realizaron con el ánimo de superar las objeciones de claridad y nivel inventivo planteadas por el examinador en su concepto técnico.

**2. Pago de tasas**

En relación con el número de reivindicaciones, atentamente nos permitimos decir que no se adjunta tasa por reivindicaciones adicionales, toda vez que el nuevo capítulo reivindicatorio tiene un menor número de cláusulas que el originalmente presentado y es en esencia el mismo objeto pero más limitado y definido con mayor claridad.

De otro lado, se adjunta recibo por concepto de pago de tasa para un nuevo examen de patentabilidad sobre el pliego reivindicatorio modificado, según lo estipulado en la Resolución No. 3719 del 2016.

**3. Claridad y concisión de las reivindicaciones**

El solicitante manifiesta que mediante la presentación del nuevo capítulo reivindicatorio quedan subsanadas la totalidad de las objeciones, de modo tal que las nuevas reivindicaciones proporcionan claridad y concisión a la invención descrita, siguiendo las recomendaciones realizadas por el examinador en su concepto técnico.

Particularmente, el examinador objetó las reivindicaciones 1-8, 13-17, 18-22, 23-26 y 27-30, afirmando que no eran concisas y que no definían los cristales reivindicados de una manera completa. El examinador requiere que el cristal se describa, por ejemplo, mediante datos de difracción de rayos X el polvo que comprendan al menos 20 picos. En este sentido, se hace notar al examinador que las reivindicaciones fueron modificadas para caracterizar el compuesto por sus picos más representativos, según el soporte que provee la Descripción.

Vale mencionar que contrario a lo que indica el examinador, no se requiere un mínimo de 20 picos. Tal como señala la Guía para el Examen de Solicitudes de Patente de Invención y Modelo de Utilidad, publicado por su Despacho, en la página 187: *"La Difracción de Rayos X es la técnica más útil para el estudio del polimorfismo en lo que atañe a la estructura cristalina, dado que los patrones de difracción de los diversos polimorfos presentan siempre*

*notables diferencias,* ***por lo que deben incluir los picos de intensidad más representativa para el caso del cristal reivindicado****"* (el énfasis es nuestro).

Por lo tanto, las nuevas reivindicaciones resultan aceptables, claras y concisas, atendiendo la solicitud del examinador.

Adicionalmente, con respecto a las reivindicaciones 1, 4, 18, 19, 22, 23, 24 y 26 (renumeradas como 1, 2 y 7-12), es importante mencionar lo siguiente:

La reivindicación independiente 1 ha sido modificada para recitar 6 picos que identifican inequívocamente la forma cristalina reivindicada. La nueva reivindicación 2 depende de la reivindicación 1 y además recita un punto de fusión, que es característico para la forma cristalina reivindicada.

La nueva reivindicación 7 es una reivindicación de composición, que recita un compuesto nuevo e inventivo y que no está limitado a formas cristalinas particulares. Por su parte, la nueva reivindicación 8, que depende de la nueva reivindicación 7, recita una forma cristalina particular y el solicitante respetuosamente considera que los 6 picos citados son suficientes para describir sin ambigüedad la forma cristalina reivindicada. La nueva reivindicación 9 depende de las nuevas reivindicaciones 7 u 8 y además recita un punto de fusión, que es característico de la forma reivindicada.

La nueva reivindicación 10 es una reivindicación de composición, que recita un compuesto nuevo e inventivo, el cual no está limitado a formas cristalinas particulares. La nueva reivindicación 11, que depende de la nueva reivindicación 10, recita una forma cristalina particular y el solicitante respetuosamente considera que los 4 picos citados son suficientes para describir sin ambigüedad la forma cristalina reivindicada. La nueva reivindicación 12 depende de las nuevas reivindicaciones 10 u 11 y además recita un punto de fusión, que es característico de la forma reivindicada.

De otro lado, el nuevo pliego reivindicatorio no incluye las reivindicaciones 2, 3, 5-8, 13-17, 20, 21, 25 y 27-30, por lo que las objeciones indicadas para ellas ya no son aplicables.

En consecuencia, nos permitimos indicar que el presente capítulo reivindicatorio, que incluye 35 reivindicaciones, se ajusta a las disposiciones del artículo 30 de la Decisión 486/00.

**4. Nivel inventivo**

El examinador considera que las anteriores reivindicaciones 1-53 carecen de actividad inventiva en vista de la Publicación de Solicitud de Patente U.S. Nº 2011/0319378 ("D1") y de la Patente de Estados Unidos Nº 8003790 ("D2").

A este respecto, me permito indicar que el artículo 18 de la Decisión 486 establece que "*Se considerará que una invención tiene nivel inventivo, si para una persona del oficio normalmente versada en la materia técnica correspondiente, esa invención no hubiese resultado obvia ni se hubiese derivado de manera evidente del estado de la técnica*".

Lo anterior significa que el análisis de nivel inventivo NO es una comparación de los objetos de las anterioridades y la solicitud en estudio. El análisis de nivel inventivo debe demostrar la obviedad de la invención y establecer cómo ésta no contiene un salto cualitativo, que demuestre su altura inventiva, teniendo en cuenta que se hace necesario demostrar que la invención analizada carece de nivel inventivo y hubiese sido obvia para una persona versada en la materia.

Considerando la anterior definición, es deseo del solicitante señalar que la combinación de las enseñanzas de D1 y D2 no hace obvia la presente invención, por las siguientes razones:

La reivindicación 1 de la presente solicitud recita la forma cristalina de un compuesto ("AMG 232") que es útil para preparar una composición farmacéutica adecuada para la administración a pacientes con cáncer. El examinador afirma que el problema objetivo técnico que la presente invención pretende resolver *"cómo modificar el compuesto conocido en el documento D1 para proporcionar el mismo compuesto con características externas diferentes"*. El examinador además sugiere que D2 supuestamente ya describe una estructura cristalina reproducible y un método para obtenerla. Se sostiene entonces que, por tanto, un experto en la técnica podría preparar la forma cristalina reivindicada como se enseña en D2, partiendo del compuesto en D1, dando como resultado una falta de actividad inventiva de la reivindicación 1.

Respetuosamente manifestamos nuestro desacuerdo con la opinión del examinador.

En primer lugar, el problema objetivo expuesto por el examinador supone que era deseable, *per se,* buscar rutinariamente formas alternativas. Se observa entonces, que el problema objetivo propuesto por el examinador ya incluye una dirección hacia la solución reivindicada, por cuanto ya incluye el aspecto del compuesto reivindicado que se encuentra en una forma diferente respecto al compuesto del Ejemplo 362 de D1. Sin embargo, el problema objetivo debe ser formulado sin dirección alguna hacia la solución reivindicada.

A este respecto, el examinador no puede ignorar que es necesario colocarse en la posición del inventor al momento de buscar una solución al problema técnico de la invención, sin partir de la invención ya conocida, porque de lo contrario, estará incurriendo en un análisis en retrospectiva, lo cual no es aceptable: *"el estado de la técnica se debe examinar* ***sin el beneficio del conocimiento ofrecido retrospectivamente por la invención reivindicada****. La indicación o la sugerencia que permita llegar a la invención reivindicada deben proceder del estado de la técnica o de los conocimientos generales de la persona del oficio normalmente versada en la materia, y no basarse en la divulgación del solicitante" (…) "En cualquier caso, el examinador de patentes* ***deberá esforzarse por hacer una evaluación práctica conforme a la realidad****. Deberá tener en cuenta todo lo que se sabe en cuanto a los antecedentes de la invención reivindicada y dar el valor justo a los argumentos o pruebas pertinentes presentados por el solicitante"* (Guía para el Examen de Solicitudes de Patente y Modelo de Utilidad publicado por su Despacho, página 138, el énfasis es nuestro).

Al momento de la presentación de la solicitud se conocía que *"[l]a forma amorfa de cualquier fármaco existe en un estado de energía libre superior [ ] respecto a su contraparte cristalina y por lo tanto proporciona una solubilidad mejorada, una velocidad de disolución más rápida y una concentración de fármaco potencialmente más alta en el tracto GI"* (véase Murdande et al., Pharmaceutical Development and Technology 16, (2011), páginas 187 a 189, columna izquierda, del cual se anexa una copia). Por consiguiente, una persona normalmente versada en la materia no se vería necesariamente motivada a buscar formas alternativas, tal como la forma cristalina reivindicada de la reivindicación 1. Incluso, asumiendo *arguendo*, que la persona medianamente versada en la materia se hubiese visto motivada a buscar alternativas, tales como formas cristalinas, es altamente impredecible si tales formas alternativas serían adecuadas para preparar una composición farmacéutica destinada a la administración a pacientes con cáncer.

Por ejemplo, al examinar únicamente las formas cristalinas, al momento de la presentación de la solicitud se reconocía que *"la capacidad de una sustancia química para adoptar más de una estructura cristalina es… un fenómeno que puede ser beneficioso y problemático"*. (véase Roy et al., Angew. Chem. Int. Ed. 48, 8505 (2009) ("Roy"), página 8505, primer párrafo, del cual se anexa una copia). Por ejemplo, Chemburkar describe el descubrimiento de una segunda forma polimórfica de Norvir después de que el producto recibió la aprobación de la FDA. (véase Chemburkar et al., Org. Process Res. Dev. 4, 413 (2000) ("Chemburkar"), del cual se anexa una copia). La segunda forma polimórfica resultó ser *"mucho menos soluble en los disolventes usados para la formulación…"* (véase Chemburkar, página 413, primer párrafo). Este descubrimiento causó problemas significativos de fabricación para Abbott y lo puso *"en una crisis de mercado"*. Por consiguiente, el solicitante sostiene que no es predecible si una forma particular, tal como una forma cristalina, **tiene propiedades ventajosas sobre una forma amorfa y cuál de varias formas cristalinas posibles es deseable**.

En segundo lugar, es bien reconocido que *"[l]a serendipia juega a menudo un papel clave en el descubrimiento de nuevas formas, porque no existe una metodología general para producir nuevas formas de un compuesto dado"*. (véase Roy, página 413, párrafo 1). Por lo tanto, *"comprender el origen del polimorfismo y controlar el resultado de los procesos de cristalización para evitar formas no deseadas…"* sigue siendo un desafío continuo. *Id.* En consecuencia, como se discutió anteriormente, **no sólo es actualmente imposible predecir si una forma cristalina particular será o no adecuada para la administración y/o fabricación de una composición farmacéutica, sino que además no existen métodos o procedimientos estándar para identificar formas polimórficas**.

La invención del solicitante es un ejemplo de libro de texto. El solicitante describe en la memoria descriptiva una multitud de experimentos diseñados individualmente y que eran necesarios para identificar una forma de fabricar y usar posteriormente la forma polimórfica específica de AMG 232 reivindicada en la presente solicitud. En las páginas 83-85 de la memoria descriptiva, el solicitante describe seis intentos de cristalizar un material amorfo, en donde todos los intentos fallaron en producir la forma cristalina reivindicada. En las páginas 84-85, el solicitante describe un tamiz polimorfo de alto rendimiento con 192 condiciones diseñadas y ensayadas individualmente que se llevó a cabo, pero no produjo la forma cristalina reivindicada. Por su parte, en las páginas 90 – 107, el solicitante describe una serie de 4

experimentos, dos de los cuales produjeron etanolato de AMG 232 cristalino. Dicho etanolato se usó entonces en el tamiz polimórfico manual descrito en las páginas 98-99 (14 condiciones diferentes), algunas de las cuales proporcionaron en última instancia la forma cristalina reivindicada. Por consiguiente, se requirió un complejo proceso de desarrollo a fin de preparar la forma cristalina reivindicada, que además demostró ser adecuada para la fabricación de una formulación farmacéutica destinada a estudios clínicos de Fase I.

Así, se demuestra que la forma cristalina descrita en la reivindicación 1 y sus reivindicaciones dependientes 2 a 6, es inventiva.

De otro lado, la nueva reivindicación 7 recita una sal DABCO de AMG 232. Este compuesto no se describe ni se sugiere por D1 o D2. Como se discutió en las páginas 122-123 de la memoria descriptiva, se demostró que la sal DABCO era un punto de control de purificación sorprendentemente eficaz, ayudando a aumentar la pureza de la sustancia farmacológica final.

Por consiguiente, la sal de DABCO descrita en la reivindicación 7 y sus reivindicaciones dependientes 8 y 9, es inventiva.

Las nuevas reivindicaciones 10 y 13 recitan sales de naftil sulfonato de AMG 232. Estos compuestos no se revelan ni se sugieren por D1 o D2. Tal como ha mencionado recientemente uno de los inventores, el Dr. Sebastien Caille, durante una presentación en el Departamento de Química y Bioquímica de la UCLA, CA, EE.UU., se encontró de forma sorprendente que la sal de naftil sulfonato es un 'guardián' de pureza eficiente, que ayuda a mejorar la pureza final de la sustancia farmacéutica. (véase Caille, S., "Amgen Chemical Process Development: A Process to Manufacture AMG 232" Conferencia de Amgen-UCLA 2016, Slide Deck, presentada el 3 de marzo de 2016, Departamento de Química y Bioquímica de UCLA, CA, EE.UU. (2016) ("Caille") en la página 14 (diapositiva 12), de la cual se anexa una copia)

Por consiguiente, las sales de naftil sulfonato de las nuevas reivindicaciones renumeradas 10 y 13, así como de sus reivindicaciones dependientes 11 y 12, son inventivas.

Las nuevas reivindicaciones 14-16 recitan una sal de sulfinato de calcio y procedimientos para hacer la misma. La sal de sulfinato de calcio no se describe ni se sugiere por D1 y D2. Adicionalmente, como explicó el Dr. Caille, los solicitantes descubrieron sorprendentemente que, bajo condiciones anhidras y temperaturas por encima de 120°C, el isopropil sulfinato de calcio, y no, por ejemplo, el derivado de magnesio, produce la sulfona deseada como producto de reacción predominante. (véase Caille en la página 18, diapositiva 16). Los solicitantes además descubrieron, inesperadamente, que el dihidrato de isopropilsulfinato de calcio, al contrario que el ácido sulfínico libre, es estable en condiciones de secado azeotrópico, lo que permite la exclusión de agua durante la reacción con el intermedio OXOS y, por consiguiente, la formación predominante del intermedio de sulfona deseado. (véase Caille, en las páginas 18 y 19 (diapositivas 16 y 17)).

Consecuentemente, las nuevas reivindicaciones 14-16 son inventivas.

Las nuevas reivindicaciones 17-23 recitan procesos de fabricación de la sal de sulfonato de naftilo AMG 232. El procedimiento no es sugerido por D1 o D2 y, por tanto, es inventivo.

Las nuevas reivindicaciones 24-28 recitan un proceso para obtener sulfona. El proceso no es sugerido por D1 o D2 y, por tanto, es inventivo.

Las nuevas reivindicaciones 29-32 recitan un proceso para fabricar una sal DABCO de AMG 232. El proceso no es sugerido por D1 o D2 y, por tanto, es inventivo.

Las nuevas reivindicaciones 33-35 recitan un proceso de fabricación de AMG 232 a partir de la correspondiente sal de DABCO. Este proceso no es sugerido por D1 o D2 y, por tanto, es inventivo.

En conclusión, la nueva reivindicación 1 y las nuevas reivindicaciones 2 a 35 son inventivas sobre D1 o D2, de forma individual o en combinación. Por consiguiente, la objeción del examinador se supera.

De esta forma, se demuestra que las nuevas reivindicaciones 1 a 35 cumplen con el requisito de nivel inventivo según los lineamientos del artículo 18 de la Decisión 486/00.

## 5. Conclusión

Con las modificaciones introducidas y los argumentos presentados puede concluirse que:

i) La presente solicitud es clara, concisa y está debidamente soportada en la descripción, cumpliendo con los requerimientos del Artículo 30 de la Decisión 486; y

ii) Las reivindicaciones actuales son patentables sobre D1 y D2, cumpliendo así con los requerimientos del Artículo 18 de la Decisión 486.

En consecuencia, se solicita atentamente otorgar el privilegio de patente solicitado, toda vez que esta solicitud cumple con los requerimientos de la Decisión 486.

Habiendo atendido cabalmente las objeciones presentadas por ese despacho, atentamente solicitamos se conceda el privilegio de patente de invención a la solicitud en estudio o si el señor examinador considera que subsisten razones para objetar la presente invención, rogamos expedir otro examen de fondo con los debidos motivos y razones que sustenten su objeción con el fin de que nuestro mandante tenga la posibilidad de exponer sus argumentos con base en la interpretación de los artículos 45 de la Decisión 486 -el cual otorga la facultad al examinador para emitir los exámenes de fondo- y 42 del Código de Procedimiento Administrativo y de lo Contencioso Administrativo que señala que: *"Habiéndose dado oportunidad a los interesados para expresar sus opiniones, y con base en las pruebas e informes disponibles, se tomará la decisión, que <u>será motivada</u>. La decisión resolverá todas las peticiones que hayan sido oportunamente planteadas dentro de la actuación por el peticionario y por los terceros reconocidos"*, (el subrayado es nuestro).

En consecuencia, solicitamos a ese Despacho pronunciarse en relación con todos los argumentos presentados en este memorial dando estricta aplicación al principio del debido proceso consagrado en el artículo 29 de la Constitución Nacional.

## 6. Anexos

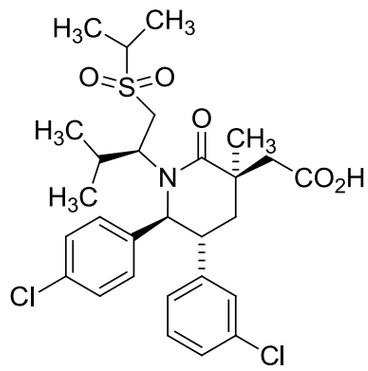
- Nuevo capítulo reivindicatorio que incluye 35 reivindicaciones.
- Copia de Murdande et al., Pharmaceutical Development and Technology 16, (2011).
- Copia de Roy et al., Angew. Chem. Int. Ed. 48, 8505 (2009).
- Copia de Chemburkar et al., Org. Proccess Res. Dev. 4, 413 (2000).
- Copia de Caille, S., "Amgen Chemical Process Development: A Process to Manufacture AMG 232" Conferencia de Amgen-UCLA 2016, Slide Deck, presentada el 3 de marzo de 2016, Departamento de Química y Bioquímica de UCLA, CA, EE.UU. (2016).
- Recibo por concepto de pago de tasa para un nuevo examen de patentabilidad sobre el pliego reivindicatorio modificado, según lo estipulado en la Resolución No. 3719 del 2016.

Señor Director,

CAROLINA DAZA MONTALVO
C.C. 66.783.790 de Palmira Valle
TP-141563 CSJ
notificaciones@clarkemodet.com.co

# REIVINDICACIONES

1. Un compuesto, en donde el compuesto es:




caracterizado porque el patrón de difracción de rayos-X en polvo comprende picos en los grados del ángulo de difracción 2-teta en 11.6, 12.4, 18.6, 19.0, 21.6 y 23.6.

2. El compuesto de la reivindicación 1, en donde el compuesto se caracteriza porque tiene un punto de fusión en 161°C.

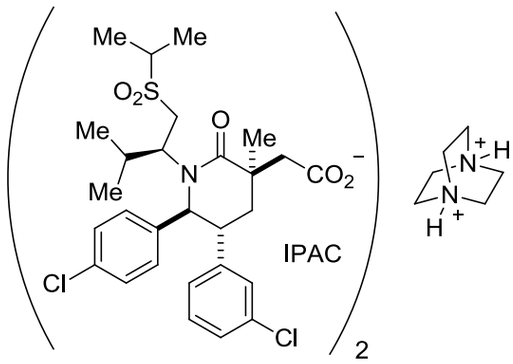
3. Una composición farmacéutica que comprende el compuesto de la reivindicación 1 y un excipiente farmacéuticamente aceptable.

4. La composición de la reivindicación 3, en donde la composición farmacéutica está en forma de una forma de dosificación sólida.

5. La composición farmacéutica de la reivindicación 4, en donde la forma de dosificación sólida es una cápsula, una tableta, polvo o gránulos.

6. La composición farmacéutica de la reivindicación 5, en donde la forma de dosificación sólida es una tableta.

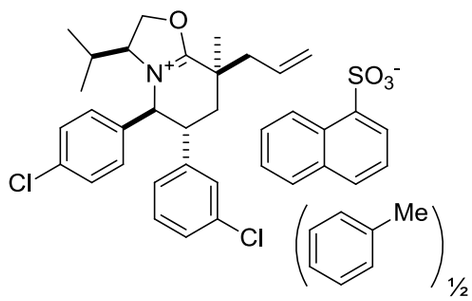
7. Un compuesto, en donde el compuesto es:




8. El compuesto de la reivindicación 7, en donde el compuesto se caracteriza porque el patrón de difracción de rayos-X en polvo comprende picos en los grados del ángulo de difracción 2-teta, en 11.5, 14.3, 15.8, 17.7, 19.5 y 20.7.

9. El compuesto de las reivindicaciones 7 u 8, en donde el compuesto se caracteriza por presentar un punto de fusión en 96°C.

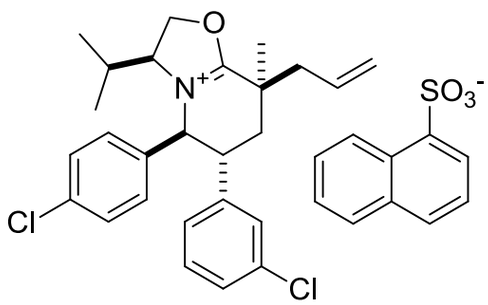
10. Un compuesto, en donde el compuesto es:



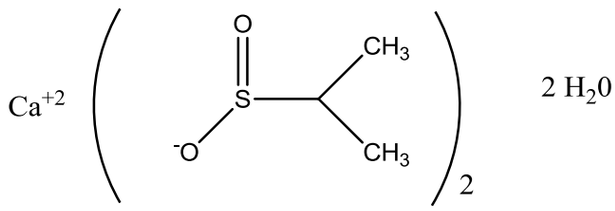

11. El compuesto de la reivindicación 10, en donde el compuesto se caracteriza porque el patrón de difracción de rayos-X en polvo comprende picos en los grados del ángulo de difracción 2-teta en 8.7, 18.5, 22.6 y 26.6.

12. El compuesto de las reivindicaciones 10 u 11, en donde el compuesto se caracteriza por presentar un punto de fusión en 208°C.

13. Un compuesto, en donde el compuesto es:



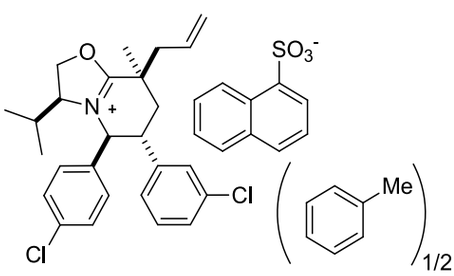

14. Un compuesto, en donde el compuesto es:



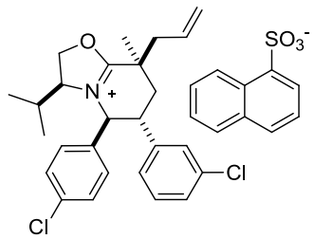
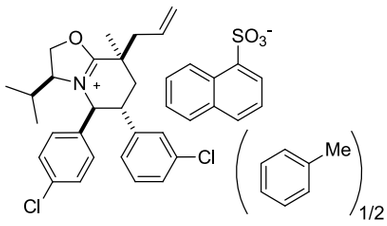

15. Un proceso para producir el compuesto de la reivindicación 14, en donde el proceso comprende hacer reaccionar ácido propano-2-silfínico con sal de calcio.

16. El proceso de la reivindicación 15, en donde la sal de calcio es sulfato de calcio.

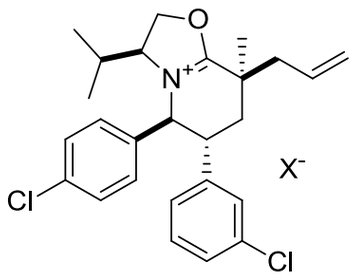
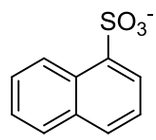
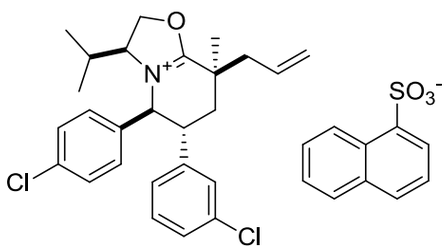
17. Un proceso para producir:

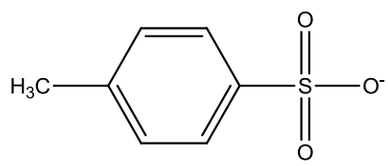


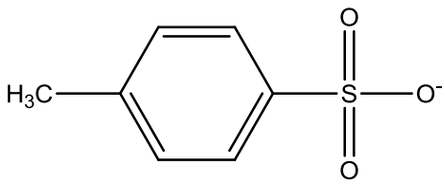

, en donde el proceso comprende:

hacer reaccionar  , con tolueno para formar  .

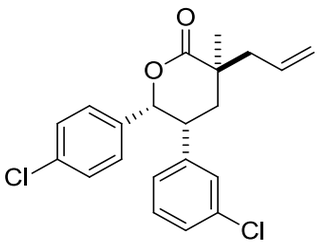
18. El proceso de la reivindicación 17, en donde el proceso además comprende hacer reaccionar:

 con  , para formar  ,

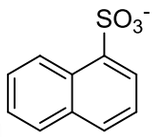
en donde $X^-$ es $CF_3SO_3^-$, o  .

19. El proceso de la reivindicación 18, en donde X es 

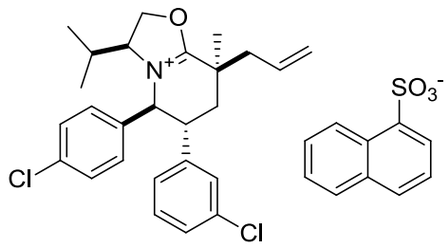
20. El proceso de la reivindicación 17, en donde el proceso además comprende hacer reaccionar:




con L-valinol, seguido de condiciones deshidratantes, y en seguida con




, para formar:

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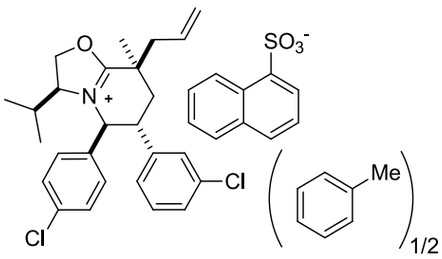
21. El proceso de la reivindicación 20, en donde las condiciones deshidratantes comprenden hacer reaccionar con anhídrido tríflico o anhídrido del ácido p-toluenosulfónico, en presencia de una base.

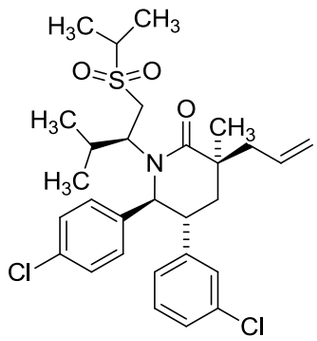
22. El proceso de la reivindicación 21, en donde la base es 2,6-lutidino.

23. El proceso de cualquiera de las reivindicaciones 17 a 22, en donde:

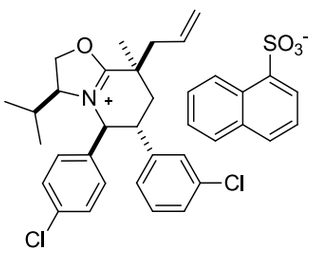



es el compuesto de las reivindicaciones 11 o 12.

24. Un proceso para producir



en donde el proceso comprende hacer reaccionar:



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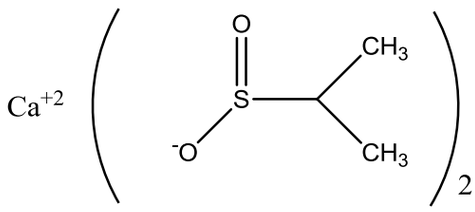

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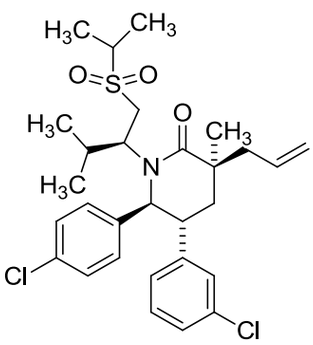
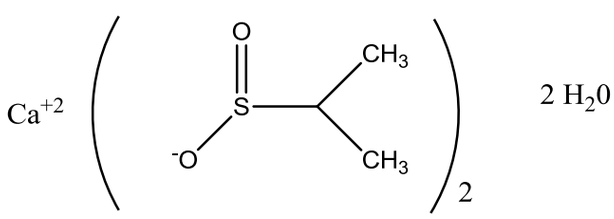
para formar:



.

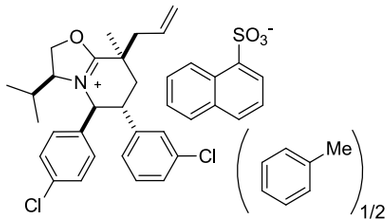
25. El proceso de la reivindicación 24, en donde el proceso además comprende

disolver



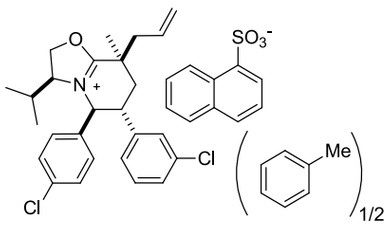
, en un solvente apropiado.

26. El proceso de la reivindicación 24, en donde el proceso además comprende

disolver 

, en un solvente apropiado.

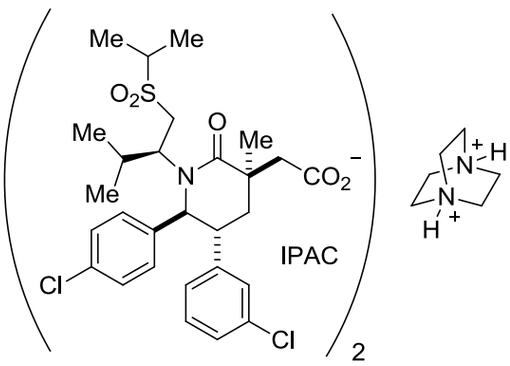
27. El proceso de la reivindicación 26, en donde:



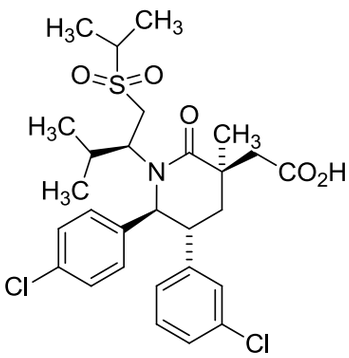
, es el compuesto de las reivindicaciones 11 o 12.

28. El proceso de cualquiera de las reivindicaciones 25 a 27, en donde el solvente apropiado es tolueno.

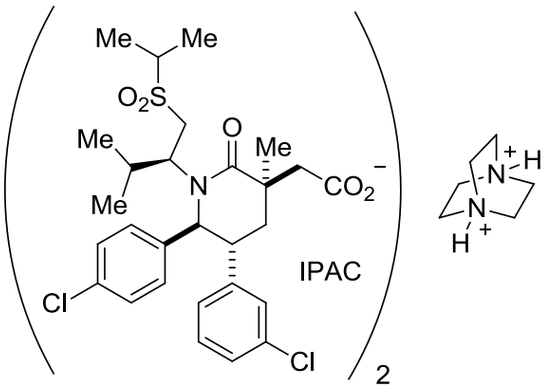
29. Un proceso para producir:



, en donde el proceso comprende hacer reaccionar:



con 1,4-diazabiciclo[2.2.2]octano (DABCO), para formar:

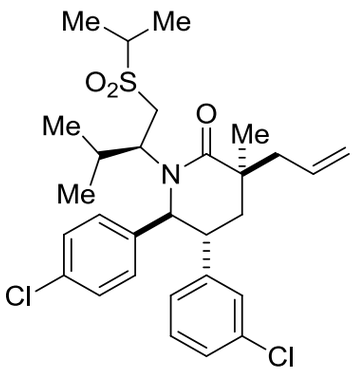




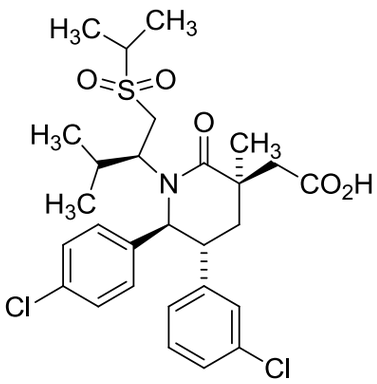


.

30. El proceso de la reivindicación 29, en donde el proceso además comprende hacer reaccionar:



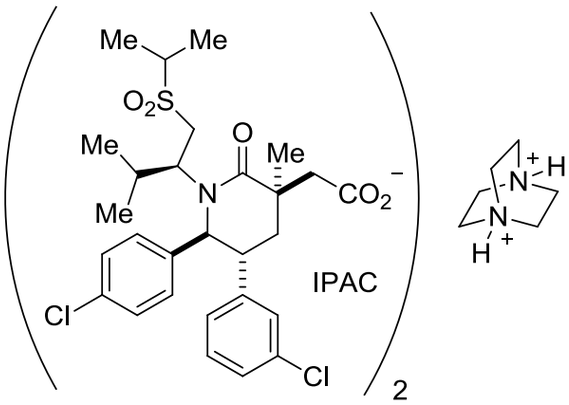

, bajo condiciones de oxidación, para formar:




.

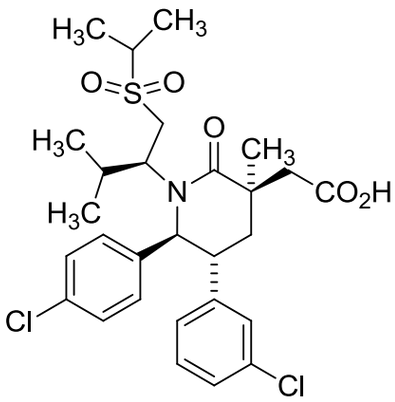
31. El proceso de la reivindicación 30, en donde las condiciones de oxidación comprenden una reacción con ozono, seguido de una oxidación Pinnick.

32. El proceso de cualquiera de las reivindicaciones 29 a 31, en donde:

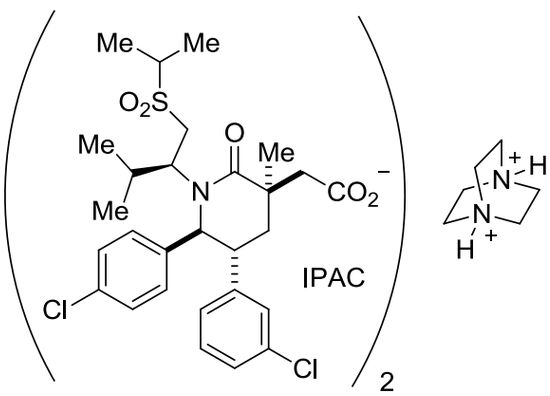


es un compuesto de las reivindicaciones 8 o 9.

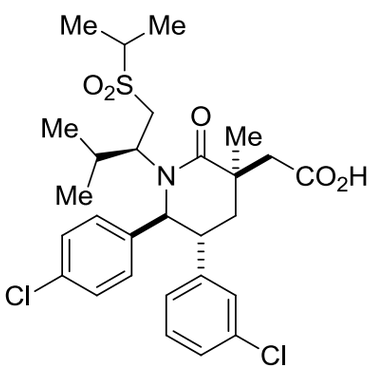
33. Un proceso para producir:



, en donde el proceso comprende hacer reaccionar:



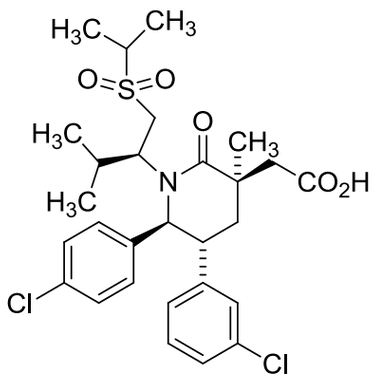
, con un ácido para formar:



.

34. El proceso de la reivindicación 33, en donde el ácido es ácido clorhídrico.

35. El proceso de la reivindicación 33 o 34, en donde:




es un compuesto de las reivindicaciones 1 o 2.

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*Pharmaceutical Development and Technology*, 2011; 16(3): 187–200

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**REVIEW ARTICLE**

# Aqueous solubility of crystalline and amorphous drugs: Challenges in measurement

Sharad B. Murdande[1,3], Michael J. Pikal[1,2], Ravi M. Shanker[3], and Robin H. Bogner[1,2]

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**Abstract**

Measurement of drug solubility is one of the key elements of active pharmaceutical ingredient (API) characterization during the drug discovery and development process. This report is a critical review of experimental methods reported in the literature for the measurement of aqueous solubility of amorphous, partially crystalline and crystalline organic compounds. A summary of high-throughput automated methods used in early drug discovery research is also provided in this report. This review summarizes the challenges that are encountered during solubility measurement and the complexities that are often overlooked. Even though there is an advantage in using the amorphous form of a drug due to its higher solubility, measurement of its solubility with useful accuracy is still a practical problem. Therefore, this review provides recommendations of preferred methods and precautions in using these methods to determine the aqueous solubility of amorphous and crystalline new molecular entities, with emphasis on the physico-chemical characterization of the solid state of the test substance.

**Keywords:** *Solubility; kinetic solubility; thermodynamic solubility; dissolution rate; amorphous; crystal; partially crystalline; high-throughput*

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## Introduction

### *General nature of the problem*

Solubility data are used to make key decisions on the developability of a new chemical entity (NCE) from the earliest stages of discovery and throughout the development process. In the pharmaceutical industry, experimental determination of drug solubility is not a single event but is commonly performed multiple times during the drug discovery and development process since synthesis and purity of active pharmaceutical ingredient (API) changes during the development process, and the assays and the tools utilized for characterization vary with the phase of development. Also the speed and quality of solubility data needed vary with the stage of drug development.

Aqueous solubility of solutes is a relatively straightforward thermodynamic concept, especially for crystalline drug molecules since solubility represents the concentration in solution in an equilibrium two phase system consisting of the drug in the solid state and the solution state. This concept is often schematically illustrated by:

$$\text{Solute}_{(\text{solid, crystalline, excess})} + \text{Solvent} \overset{K}{\Leftrightarrow} \text{Solute}_{(\text{solution})}$$

where K, the equilibrium constant, is the ratio of activity of solute in solution to that in the solid. This equilibrium constant is equal to the solubility in cases of practical interest where solute concentrations are small and solute activity coefficients are essentially unity.

For practical purposes, solubility is defined here as the concentration of the solute in solution which is in equilibrium with solute in the solid state at constant temperature and pressure. Thermodynamic (equilibrium) solubility represents the saturation (i.e. maximum, time independent) concentration of a compound in equilibrium with an excess of undissolved solid phase. Of

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course, solubility only has meaning if the nature of the solid sample is known, as changes in solid-state form or composition – either before or during the measurement – will alter the equilibrium concentration, and therefore, change the solubility.

Classical solubility measurements are based on variations of the shake-flask method.[1,2] In practice, equilibrium solubility is often determined only by a single concentration measurement made after a time assumed to be sufficiently long for equilibrium to have been established, generally after an equilibration time of 24–48 h. However, to confirm that equilibrium has been achieved, it is desirable to measure the concentration of drug in solution at multiple time points to demonstrate that the concentration is indeed invariant with time. Additionally, characterization of the remaining excess solid, to preclude the possibility of any on-going transformation, is essential to confirm the nature of the equilibrium state. Approaches to accelerate the attainment of equilibrium for compounds with low aqueous solubility have also been reported.[3] Important practical precautions that must be taken when measuring solubility include:

(a) Ensuring high chemical purity of solute and solvent;
(b) Selecting satisfactory techniques for complete separation of the saturated solution from undissolved particles;
(c) Employing appropriate analytical methods to quantify drug concentration in solution, including the ability to detect any decomposition during measurement;
(d) Controlling the temperature;
(e) For aqueous solutions, measuring the pH of the solution at the end of equilibration period; and
(f) Analyzing/characterizing the excess solid separated from the saturated solution to determine whether any changes in solid phase may have occurred relative to the initial starting solid.

While the concept is simple, the actual assessment and prediction of aqueous solubility represent both experimental and theoretical challenges. Determination of drug concentration in solution, especially with the use of automated HPLC procedures is a simple matter, but the concentration obtained by assay is often highly dependent on how separation of solute and solvent is achieved. Therefore the pragmatic formulation scientist needs to be cognizant of the experimental factors that influence the determination of equilibrium solubility. Factors such as: (a) Time required to attain equilibrium, (b) confirmation of attainment of equilibrium, (c) the nature of drug species present in solution at equilibrium, (d) identity of other components present in aqueous media and their influence on drug species in solution, (e) temperature of the system and impact of temperature errors[1], and (f) degree of solute crystallinity, purity and the necessity of isolation and characterization of the solid form of drug at equilibrium, i.e. polymorph, hydrate, solvate formation and others. Experimental factors such as rate of agitation or mixing and control of temperature influence powder dispersion and the time required for attainment of equilibrium, and therefore may impact solubility measurement. All of these and other factors are the subject of several reviews[4,5] and books.[6,7]

The crystalline state is classified into 230 unique space groups (based on 14 Bravais lattices and 32 unique point groups that are arranged in seven 3-dimensional coordinate systems). The existence of multiple crystalline forms (polymorphs, hydrates, solvates and co-crystals) of drug molecules has been extensively reported and reviewed.[4,8] Despite their 3-dimentional long range order, it is still not possible to *a priori* predict the identity of all potential polymorphs and their corresponding solubilities for any crystalline drug.[8–10] The quest for predicting polymorphic forms of organic, molecular crystals from their structure is an area of active research and considerable progress is being made using computational methods.[11,12] Even nominally crystalline forms can present problems in accurate solubility measurements. Organic molecular crystals are well known to exist in multiple polymorphic or pseudo-polymorphic forms, and Figure 1 depicts potential transformations that can occur either via solution or solid state that result in physical form changes of the crystalline drug. Obviously, if the solid undergoes a phase transformation during the measurement, then the concentration in solution is not related to solubility of the starting solid phase. Anticipation of the existence of various metastable crystalline forms or the *a priori* prediction of the thermodynamically most stable form is far from an exact science.

One of the frequently used approaches to identify new polymorphic or pseudo-polymorphic crystalline forms is through solubility studies. The potential transformations shown in Figure 1 also illustrate the importance of: (a) Creating experimental conditions such that equilibrium between solid and solvent is established, and (b) utilizing appropriate analytical techniques to characterize the form of the solid present at equilibrium. In Figure 1, the kinetics of conversion from an initial crystalline form to a new form is represented by a positive numerical subscript (e.g. $k_1$). The reverse process, i.e. conversion of new crystalline form to the initial form is represented by a negative subscript (e.g. $k_{-1}$). Thus, Figure 1 also emphasizes the importance of rates of the various interconversions and the importance of providing adequate time to

[1] Using a heat of solution value of 10 kcal/mol, which is typical for most organic compounds, a temperature control of ± 1°C is required to measure solubility value with ± 10% accuracy, and by proportion, 1% accuracy in solubility would require ± 0.1°C temperature control.

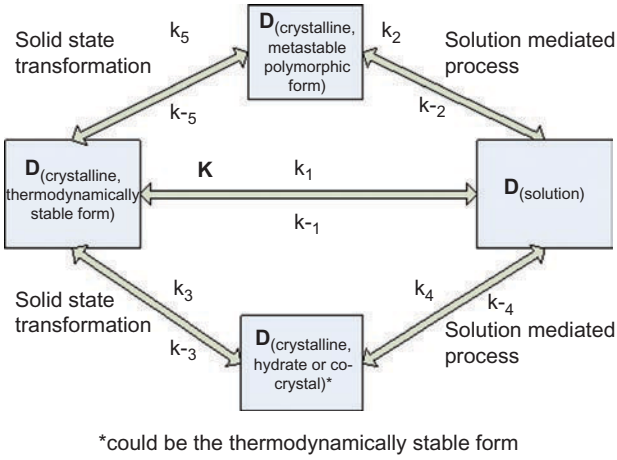


**Figure 1.** Pathways for transformations of crystalline, non-ionizable drug.

reach equilibrium, assuming any chemical degradation is negligible. Since many of the conversions depicted in Figure 1 are solution-mediated processes, these transformations could also occur during the measurement of solubility of partially crystalline as well as amorphous drug forms. Such transformations, if not monitored by analyzing the properties of the excess solid, could result in highly variable and erroneous estimates of solubility of crystalline, partially crystalline or amorphous drugs. To date, instrumentation and methodologies that have been used to characterize solid state properties of pharmaceutical materials have largely been developed for crystalline materials. Techniques such as x-ray diffraction (powder and single crystal), spectroscopy (FTIR, NIR, Raman), thermal analysis (DSC and MDSC) and solid state NMR have all been demonstrated to have the ability to at least qualitatively differentiate among multiple solid state crystalline forms of pharmaceuticals.[4,13] The ability of these techniques to provide quantitative information about the relative abundance of two or more crystalline forms in a sample is typically reliable and reproducible at levels > 5%.[14,15] However quantification at levels less than 5% and more importantly at levels less than 1% is dependant on the properties of the compound and the sensitivity of the specific technique for the compound of interest. Moreover, quantification of the degree of crystallinity in mixtures of crystalline and amorphous forms in complex formulations poses serious difficulties, particularly in systems with amorphous or partially amorphous excipients.[16,17]

The amorphous form of any drug exists in a higher free energy state[5] than its crystalline counterpart and therefore provides enhanced solubility, faster dissolution rate and potentially higher drug concentration in the GI tract. This feature has attracted the attention of many pharmaceutical researchers and has been exploited by formulation scientists to enhance the oral bioavailability of insoluble compounds.[18–22] However, the assessment of the potential solubility advantage of amorphous drugs remains a challenge. The research activity over the past decade exploring and understanding the properties and performance of amorphous pharmaceuticals, suggests there are three major challenges in the development of amorphous materials for oral drug delivery applications.[23,24] These are (a) the development of meaningful characterization tools for amorphous materials; (b) bioavailability prediction; and (c) the engineering of custom materials to stabilize the thermodynamically unstable form both in the solid state and during dissolution. Experimental approaches that would allow better characterization of the amorphous drug may facilitate its widespread use of amorphous solids in drug delivery applications.

Rigorously speaking, the term solubility is only associated with equilibrium conditions. With a pharmaceutical glass, one does have a non-equilibrium system from several perspectives: (a) Chemical instability, (b) changes in free energy due to relaxation effects, and (c) the tendency to crystallize during the dissolution or solubility experiment. Since chemical instabilities for most amorphous drugs normally occur on a time scale that is long compared to the time required for solubility measurement, such instabilities are not usually a problem regarding solubility determinations. Relaxation effects can occur on much shorter time scales, and in principle could produce small differences in free energy between samples chemically identical but with different thermal histories[2].[25] However, due to plasticization of the amorphous solid by water during the attempted solubility measurement, the relaxation time will be greatly decreased, and in most cases the resulting faster relaxation would bring all samples to nearly identical state well before mass-transfer equilibrium is established during the solubility measurement. Therefore, the real non-equilibrium problem with amorphous solids is crystallization from the super-saturated solution. Crystallization is frequently a serious problem, and if crystallization does occur before a 'plateau' in concentration is reached during a dissolution-based solubility measurement, the 'peak' concentration is most certainly less than the expected solubility of the amorphous form, and often much less! However, unless specifically noted otherwise, we use the term, 'apparent solubility' to describe the maximum solution concentration obtained during dissolution of an amorphous form even if a prolonged period of constant concentration is not observed, realizing that in such cases the 'apparent

[2] Differences in thermal history do produce differences in heat capacity and enthalpy, and therefore also in free energy and solubility, but relative to the difference between the given amorphous state and the crystalline state, the impact of thermal history variation on free energy of the amorphous state is expected to be quite small.

solubility' is less than the thermodynamic solubility of the amorphous form.

Formulators need a reliable way to measure the maximum concentration the amorphous drug can provide before they spend the time trying to achieve it using a formulation. Although the concept of solubility requires assessment of the state of the drug in the solid state at equilibrium, the literature on amorphous solids is focused on methodologies for quantification of drug in solution and does not always discuss characterization of the solid in contact with the aqueous system. We review the literature reports on measurement of aqueous solubility of crystalline, partially crystalline and amorphous solids. This report also reviews methods that have been used to assess the solubility of amorphous solids in multi-component pharmaceutical dosage forms, as for example, with the amorphous drug stabilized using a carrier excipient.

## Terminology

Terms such as 'apparent solubility',[26] 'apparent equilibrium solubility'[27] and 'kinetic solubility'[28–33] as well as measures of solubility using intrinsic dissolution rate have been introduced to describe the dissolution profiles of amorphous and partially crystalline drugs in solution. The term 'apparent solubility' was provided by Mosharaff et al.[34] to refer to the persistently high concentration of solutions prepared from metastable materials. The authors rationalized the persistence of supersaturated solutions by postulating the presence of an outer particle layer disordered by mechanical stress which increases the 'apparent solubility'.[27] The term 'apparent equilibrium solubility' was defined as the concentration of the solution in apparent equilibrium with the solid, where the qualifier, 'apparent', is inserted because the solid is not in its most stable state,[27,35] and in many cases, the concentration in solution is not even independent of time over a significant time period. The high apparent equilibrium solubility of co-ground amorphous indomethacin observed to persist for at least 40 min in Watanabe's study[35] was attributed to short range disordering in the samples, which may be a valid conclusion, but the main point is that in such cases one is not dealing with an equilibrium system, even over the time period relevant to the experiment. Even if a concentration independent of time is observed over a substantial time period, such a concentration is not rigorously an equilibrium property because the amorphous state is not the equilibrium solid state. However, one must remember that no systems that are dealt with in the pharmaceutical industry are truly equilibrium systems if all types of potential changes are considered, including physical and chemical stability.[36] For example, a mixture of hydrogen and oxygen is not an equilibrium system with regard to chemical reaction, but yet one may apply the usual laws of thermodynamics as long as the composition of the system is independent of time over periods relevant to the problem under discussion.

To further complicate the terminology of the field, 'kinetic solubility' which is traditionally used to describe the concentration from a measurement described by Lipinski,[37] it has also been used to describe the peak concentration obtained during a dissolution experiment.[31] Additionally, Bahl and Bogner[38] used the term 'maximum transient concentration' to describe the same peak. It would be useful to come to a common terminology. The most common terminology appears to be 'apparent solubility'. While this term offends many purists, it serves as compact notation for the peak in a concentration-time profile for dissolving solids. The term 'apparent equilibrium solubility' should be reserved for such concentrations that persist for a substantial period of time. We can consider the eventual use of the solid to determine what is a "substantial period of time". The clinical use provides a benchmark. For oral solid dosage forms, the roughly 3- h small intestinal transit time is a reasonable time frame over which one would desire a concentration to persist. For nanoparticles that are intended for intravenous circulation, perhaps a longer time period would be appropriate. In any case, it would be helpful for authors to explicitly state the period over which the observed 'apparent equilibrium solubility' values persist.

## Solubility measurement methods

The approaches to finding the aqueous concentration in equilibrium with a solid can be categorized into two types: (1) Precipitation from supersaturated solutions, and (2) dissolution from the solid. In addition, there are methods that cycle between the two.

### *Precipitation from supersaturated solutions*

The concept of solubility measurements based on precipitation from supersaturated solutions has been introduced in the literature, especially for early physico-chemical characterization studies of new chemical entities. Such studies are needed when small quantities of large numbers of potential drug molecules are synthesized for evaluation of structure activity relationships, physico-chemical properties (including solubility) and metabolic profiling. A turbiditmetric solubility method was first introduced to the pharmaceutical industry by Lipinski et al.[37] to meet the needs of the earliest stages of drug discovery and development. The type of solid state material that is present during this stage can be amorphous or crystalline, non-ionizable or salt form, co-crystal or a combination of these possibilities. The Lipinski turbiditmetric method uses highly concentrated solutions of drugs prepared in a

water miscible solvent (most often DMSO) which are then added to a well stirred aqueous medium until precipitation occurs in the largely aqueous medium, regardless of what solid form precipitates. The appearance of a precipitate is detected by monitoring turbidity of the aqueous solution upon incremental increase in the amount of DMSO stock solution to the aqueous media. This method is used to rank or classify molecules to determine whether they meet a desired solubility value. The authors recognized the limitations of their approach; the methodology is meant to be quick and is meant to provide a useful rank order but not necessarily an accurate estimate of aqueous solubility. In the precipitation methods which employ a co-solvent, such as DMSO, solubility values obtained are likely to over-predict the true thermodynamic solubility, due to the degree of supersaturation that may occur, and are likely not even reproducible as details of the method are altered. In the study reported by Stuart and Box,[39] the solubility values determined by a similar so-called 'kinetic procedure' were much higher than the equilibrium solubility values, and in cases where a compound may attain a high degree of supersaturation, the 'measured' solubility was up to 50 times the true thermodynamic solubility. Therefore, solubility values determined by 'kinetic' or precipitation approaches cannot be assumed to represent a close approximation to the equilibrium solubility of a compound, and rank order of 'solubility' measured for a series of samples may not even be correct. However, the speed of 'kinetic solubility' measurement has provided a tool to obtain a solubility estimate for a large number of compounds in a relatively short period of time. The resulting data have been utilized to rank order compounds in a structural series during the process of discovering new chemical entities against a specified target.

### *Dissolution-based methods*

In contrast to precipitation techniques, procedures based upon measuring dissolution (i.e. concentration-time profile) using drug powder have been more common for estimating the solubility.

#### Powder dissolution

The shake flask method is a type of dissolution method that is based on the phase solubility technique developed by Higuchi and Connors[40] and is the gold standard for thermodynamic solubility determination.[41] The method can be divided into five steps: (1) Sample preparation, (2) equilibration, (3) separation of phases, (4) analysis of the saturated solution, and (5) characterization of the residual solid. The assay is performed by adding an excess amount of solid to the solubility medium and mixing for long periods of time, typically 24–48 h. To speed up the equilibration process, samples can be vortexed or sonicated prior to equilibration. The solution is then filtered and the supernatant is diluted and assayed using an appropriate assay, such as HPLC or UV-Vis spectrophotometry. As with any solubility determination, it is necessary to control the temperature of the system to about ± 0.1°C, and it is critical to ascertain whether the solid phase of the solute changes during equilibration to produce different polymorphs or solvates by analyzing the solid phase, typically using x-ray powder diffraction. The traditional shake flask method can provide accurate thermodynamic solubility values and is often used as the standard method against which other methods are compared. However this method is slow, labor intensive, and not easily adapted to the needs of high-throughput screening (HTS) and early stages of drug discovery.

An alternative method to the shake-flask technique for solubility measurements is the combination of precipitation and dissolution techniques described by Avdeef and Berger.[42–44] The method is intended to yield thermodynamic solubility but is only applicable for the determination of solubility of the non-ionized form of a compound with ionizable groups (i.e. neutral species). The method involves titrating a basic compound from high to low pH, or an acidic compound from low to high pH, where pH is the measured response. The presence of precipitate in the course of titration causes the apparent ionization constant ($pK_a^{APP}$) to shift to higher values for acids and to lower values for bases. The precipitation causes the titration curve to shift in the region of the $pK_a$ and $pK_a$ appears to be larger than true pKa ($pK_a^{APP}$) for a weak acid. The procedure involves using a Bjerrum plot (i.e. plot of average number of bound hydrogen ions versus pH). These plots reveal true and apparent pKa. The solubility of the neutral species is determined by applying the relationship:[42,45]

$$\log S_0 = \log\left(\frac{C}{2}\right) - \left|pK_a^{APP} - pK_a\right| \quad (1)$$

where C is the sample concentration and $S_0$ is the intrinsic solubility (i.e. the solubility of the non-ionized form of the drug). In order for equilibrium to be established, a typical titration takes 3–10 h to complete, depending upon the solubility of the compound, compared to the 48–72 h recommended for classical shake-flask method. The rate of data collection is slowed dramatically near the regions of complete dissolution as the time taken to dissolve additional solid increases significantly at this point. In this method the solid form at equilibrium is not characterized so no information is obtained on which solid form the 'solubility' value reflects.

In another study by Avdeef et al.,[44] the titration method was validated against a saturation shake-flask method using a set of 12 compound that ranged in solubilities over six orders of magnitude. These authors found

a good correlation ($r^2 = 0.98$) between intrinsic solubility values obtained by these two techniques.

**Intrinsic dissolution**

In principle, solubility may also be determined by intrinsic dissolution rate measurements. The intrinsic dissolution rate has been described as a 'particle size independent' measurement of the concentration-time profile of a drug powder that is usually obtained by producing a solid compact of known surface area and measuring its dissolution rate under conditions of controlled and generally well understood hydrodynamics. Because the area of the flat surface does not change with time, the dissolution rate depends on the solubility of the solute, rotation speed, and mass transfer coefficient of the drug in the dissolution medium.[6,46] With its well characterized fluid hydrodynamics, the classical disk intrinsic dissolution rate (IDR) can be used for determining the rank order of solubility of compounds that do not 'react' (i.e. ionize, complex or otherwise interact) with the dissolution medium.

Intrinsic dissolution studies are carried out with a rotating disk of compacted drug powder that is immersed in the dissolution test medium (Wood's method).[46,47] In the common modifications of the disk intrinsic dissolution studies (Wood's method), about 150–700 mg of pure drug compound is compressed in a punch and die to produce a pellet with an exposed surface area typically from 0.5–1.3 $cm^2$.[48,49] The feasibility of using intrinsic dissolution rate measurements to determine placement in the Biopharmaceutics Classification System (BCS) solubility class was investigated by Yu et al.[48] The dissolution method was suggested to be a simple and convenient way to classify the BCS solubility of drugs. However, this is only strictly true for compounds that do not ionize or complex during dissolution; in those cases, the intrinsic dissolution rate of small compounds will be linearly related to solubility. However, when compounds ionize or others interact with the dissolution medium, the dissolution rate is a more complex function of solubility, dissociation constant(s), concentration of 'reactive' species in the dissolution medium, and flow rate of the dissolution medium.[49–53]

### *Combination of precipitation and dissolution-based methods*

Stuart and Box[39] have described an alternate method, called 'chasing equilibrium' for measuring intrinsic solubility of acids, bases and ampholyte compounds. In this procedure, a quantity of compound is completely dissolved at a pH where it exists predominantly in its ionized form, and then a precipitate of the non-ionized (neutral) species is formed by changing the pH. Subsequently, the rate of change of pH due to cyclic precipitation and dissolution is monitored and a strong acid (or base) titrant is added to adjust the rate of pH change to zero, which defines the pH at which equilibrium exists between dissolved (neutral) solute and solid. Thus, the solubility of the neutral form of the compound may be calculated from the equilibrium pH, the pKa and the total solute concentration. The term, 'chasing equilibrium' refers to the practice of repeatedly changing from supersaturated to underaturated and back again by changing the pH. At least in principle, the method provides equilibrium solubility data. The basis of the method involves tracking pH changes induced by precipitation and seeking an equilibrium pH where sample is neither precipitating nor re-dissolving. The occurrence of precipitation is detected using a spectroscopic dip probe. This solubility determination takes 20–80 min per sample. The rapid determination of equilibrium solubility, as compared to the traditional shake-flask method, is due to precipitation of neutral solid caused by rapid change in pH condition. At least in principle, equilibrium solubility may be obtained since equilibrium is being approached from both below and above the saturation. This process does not require separation of solid from the solution and hence the method avoids a filtration or centrifugation step that would be required in standard equilibrium solubility measurements. There is a lower limit of 2–5 mg of compound required for the method to produce reliable results regardless of the solubility and ionization properties of the molecule. An example of equilibrium chasing is shown in Figure 2 for a weak acid drug, diclofenac.

At the start of the assay, information about the molecular weight of the drug, weight of the sample added into

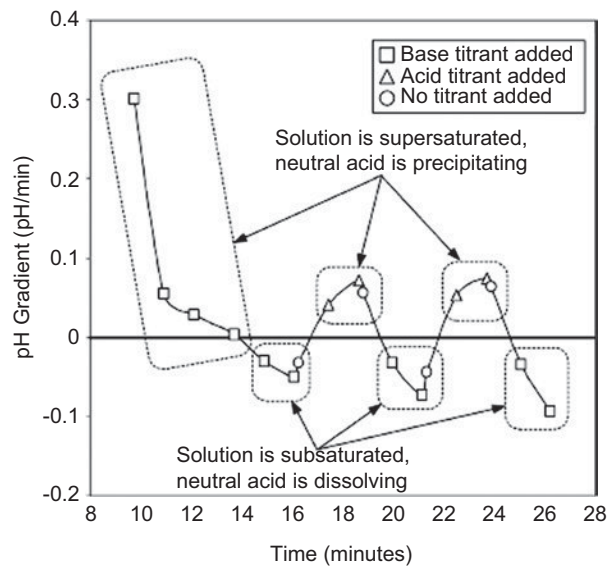


**Figure 2.** Change of pH gradient while chasing equilibrium of the weak acid drug diclofenac, showing five changes from supersaturation to subsaturation and vice versa. Figure adapted from reference [37]. (Reproduced with permission from Analytical Chemistry, ACS Publications).

the titration vessel, concentration of acid and base titrants and ionization constant(s) of the drug are needed. The experiment proceeds in five stages, (1) Dissolution, (2) seeking precipitation, (3) additional precipitation, (4) chasing equilibrium, and (5) re-dissolution. For an acid drug, adding base titrant increases the pH and reduces the concentration of neutral species without having to wait for more of the drug to precipitate. Similarly, adding acid titrant rapidly increases the concentration of the neutral species without having to wait for more dissolution. After precipitation of the neutral species occurs, the solution is *repeatedly* changed from supersaturated to undersaturated and back again by changing the pH. Typically this stage is repeated until around five to eight saturation state changes are measured, as shown in Figure 2. The concentration of non-ionized species for a monoprotic acid in solution is determined by the following equations:

$$[HA] = \frac{[A^-][H^+]}{10^{-pKa}} \tag{2}$$

$$[A^-] = [H^+] - [OH^-] + [K^+] - [Cl^-] \tag{3}$$

$$S \approx [HA] \tag{4}$$

where [HA] is the concentration of the non-ionized species in solution which is equal to its intrinsic solubility (S), $[A^-]$ is the concentration of the ionized species of the drug, $[K^+]$ is the concentration of base titrant used and $[Cl^-]$ is determined from the amount of acid titrant.

In another study, Box et al.[54] distinguished two classes of compounds, chasers and non-chasers, based on whether a compound chases equilibrium or not. Compounds that do not form supersaturated solutions as acid (or base) titrant is added to convert the molecule to its non-ionized form, fall directly out of solution as soon as the pH is reached where the compound exceeds its saturation limit. Thereafter, as more of the titrant is added, the pH reading reaches a stable value quickly after each addition of titrant. These compounds do not chase equilibrium in the same way as compounds that have the ability to form supersaturated solutions. Therefore, compounds that form supersaturated solution are called 'chasers' and that do not form supersaturated solution are called 'non-chasers'. The solubility determination process is much shorter for 'non-chasers'.

While the combination of precipitation and dissolution described above is a relatively fast method and at least potentially can provide accurate thermodynamic solubility, the methods has two significant limitations: (a) The method is suitable for only ionizable compounds, and (b) the solid form at equilibrium is not characterized so no information is obtained on which solid form the 'solubility' value reflects. This limitation would be particularly serious for new chemical entities where the propensity for polymorphism is high and the thermodynamically most stable form is unknown. That is, one would not know whether the solubility refers to the most stable solid form or some metastable form. The classical shake-flask method would be most suitable in this case, where one can identify the solid present at equilibrium between the solid and the solution. Box et al.[54] validated their method for intrinsic solubility determination against the classical saturation shake-flask method using a small set (16 compounds) of organic compounds covering wide range of solubility (2–6000 μg/ml). They observed a good correlation between solubility values obtained by the two techniques ($r^2 = 0.96$).

### *Adaptations to high-throughput methods*

High-throughput solubility assays are often based on the detection of precipitation of compounds from aqueous buffer solutions as described in the section entitled *Precipitation from supersaturated solutions*. Typically, small volumes of the stock solution, containing high drug concentration in an organic or mixed water-organic solvent system, are added incrementally to the aqueous (buffer) solution of interest until the solubility limit is reached.[37] The resulting precipitation is detected optically, and the apparent solubility is defined as the concentration preceding the point at which precipitation occurs. In the original procedure described by Lipinski et al.,[37] DMSO stock solutions are added in small aliquots to the aqueous solutions, spaced a minute apart, until precipitation occurs. Alternatively, a series of specific concentrations may be prepared by dispensing the appropriate volume from a DMSO stock solution into separate vials.[55] In both set-ups, the amount of DMSO in solution increases with drug concentration and will cause a higher solubility than would be observed in pure water, with DMSO operating as a cosolvent. There are two popular experimental approaches for evaluating solubility in high-throughput mode. One involves removing the precipitate and determining the concentration of compound in solution and the other using the precipitation event itself as an indicator for the solubility limit of a compound; that is, the calculated concentration in the clear solution immediately before precipitation is detected is taken to be the solubility.

There are numerous methodologies for determination of precipitation, such as turbidimetry/nephelometry,[28,37,56] absorption spectrometry[45,57,58] and the modified equilibrium method.[57] Methods like nephelometry[28] and absorption spectrometry are suitable for high-throughput solubility determinations using commercial 96-well plates. Another automated approach to couple solubility with final pH and crystallinity for use

in an early pharmaceutical discovery setting is described by Seadeek et al.[59] Their automated workflow station is based on a 96-well plate format, robotic liquid handler and UV-Vis plate reader. This nephelometric method is performed using serial dilution in aqueous buffer. A plot of the intensity of light transmission vs. compound concentration indicates the concentration at which the compound starts to precipitate, which is taken as the solubility. A robotic liquid and plate handler coupled to nephelometric detection for precipitation measurement of solubility is also described by Dehring et al.[60] Another high-throughput solubility assay incorporates determination of crystalline precipitate utilizing polarized light microscopy.[61]

It should be emphasized that, with all such precipitation methods, compounds that do not precipitate as well defined crystalline solids will likely give apparent solubility values far in excess of the thermodynamic solubility of the crystalline form. Thus, all of the above automated high-throughput solubility measurement methods do not necessarily measure thermodynamic solubility or anything close. Speed of measurement, or high throughput, has its price. Limitations include: (a) The form in which the drug precipitates is not identified and may not even be crystalline, (b) due to supersaturation, measured values tend to be high, (c) shifts in pH value during the measurement and impurities are not detected and (d) drug decomposition during solubility measurement is unknown. These limitations can easily lead to estimates of solubility that are much higher than the actual thermodynamic solubility of the crystalline form of interest. Therefore, the pharmaceutical industry faces the dilemma of accepting automated high-throughput solubility measurement methods which provide the speed that satisfies the needs of drug discovery or to use the much more accurate, but more time consuming, traditional equilibrium solubility (e.g. shake-flask) measurement methods.

Recently Wyttenbach et al.[64] have reported a miniaturized method for solubility and residual solid screening of drug compounds in aqueous and non-aqueous vehicles for early drug development. This method does have high-throughput characteristics since a 96-well system is used, but has several significant advantages relative to the high-throughput methods described above. Experiments are performed in 96-well multi-screen solubility filter plates. The bottom of the plate is sealed with adhesive aluminum foil to prevent evaporation of the solvents during equilibration. The compound (10–15 mg solid per well) is dispensed into filter plates and 100 μL of aqueous medium is added. Immediately after aqueous medium addition, the plate is sealed by a septum sheet. The drug-aqueous medium slurries are then mixed by over-head rotation at 20 rpm for 24 h at room temperature. At the end of the 24-h period, the liquid is separated from the residual solid by centrifugation. The filtrates are then analyzed by UPLC (ultra-performance liquid chromatography), and the wet solid residue left on the filter plate is analyzed by high-throughput transmission XRPD. This method allows determination of both solubility and the solid form in equilibrium with the solution, although the technique does not rule out contamination by the amorphous phase. Recent reports in the literature[63–65] have also shown the utility of such techniques for screening polymers that prevent or slow the kinetics of crystallization of insoluble compounds from supersaturated aqueous solutions. These approaches are being advanced to assist in the development of amorphous solid dispersions. This method comes closest to the classical shake-flask method in the ability to determine thermodynamic solubility. However even the relatively long equilibration time of 24 h used in the Wyttenback method may not be adequate for particularly poorly soluble compounds that dissolve very slowly.

A small-scale automated solubility measurement apparatus was also reported by Yi et al.[66] that operates non-isothermally. It consists of a 'reaction' station, turbidity probes, light source and a chiller. The solubility protocol calls for six samples of slurries with different solute amounts placed in the reaction chamber of the apparatus. The temperature of each sample is increased 1°C at a time and held at that level for up to 60 min. This procedure is repeated until all crystals in the sample disappear. The disappearance of the crystals is detected by fiber optic turbidity probes. The temperature at which all crystals in a sample dissolve is determined from the transmission versus temperature profile. The solubility data obtained with this procedure for three test compounds showed agreement (within 5%) with literature values.

## Impact of amount of excess solid on solubility measurement and dissolution

Often, when a solubility value for any compound is reported, the amount of excess solid used in the experiment is not mentioned since in principle, at equilibrium the amount of excess solid should not influence the assessment of solubility. However, in practice, the solid is often a mixture of amorphous and crystalline phases, which means the measured solubility may be well in excess of the equilibrium solubility of the crystalline phase when a large excess of solid is used; that is, the solubility measured is essentially due to dissolution of the amorphous phase and the failure of the resulting super-saturated solution to crystallize. However, other anomalous effects of excess solute on the solubility of drugs have been reported. Wang et al.[67] reported that solubility of a dihydrochloride salt of a diprotic weak base depended on the amount of excess solid used in the experiment. In Wang et al.'s study, varying the

amount of excess solid (a) was demonstrated to influence the concentration of the counterion, chloride, and thus suppress the solubility of the diprotic weak base via common-ion effect, and (b) this impact was observable only in the pH region in which monohydrochloride salt was expected to be the solid form at equilibrium. When the solubility determination was performed in the pH region 2–5 where monohydrochloride salt determines the solubility, the excess amounts of dihydrochloride salt converted to monohydrochloride and released chloride ions that suppressed the solubility via common ion effect. Significant differences in the two $pK_a$s (3.1 and 7.7) led to differences in solubility in the pH region 2–5 depending on the amount of solid added. Therefore this effect was not an intrinsic effect of excess solid on solubility but was due to the physico-chemical property (large difference in the two pKas of the diprotic weak base) of the drug.

In contrast, Kawakami et al.[26] investigated the effect of excess solid on the apparent solubility of three free acid forms of drugs as well as one basic drug at various pH conditions. In order to understand the dependence of solubility on the amount of solid used in the experiment, the authors evaluated the dissolution and crystallization rates of indomethacin at near-equilibrium conditions. The authors conducted dissolution studies by preparing suspensions by adding the desired amount of indomethacin in buffered solutions at pH 5 and pH 7 two days prior to the experiments, where the amount of drug added in the suspension was systematically varied. At pH 5 the drug levels investigated were 40, 200 and 5000 μg/mL and at pH 7 the levels were 1500, 2000 and 10,000 μg/mL). Figure 3 shows time profiles of the concentration of the dissolved indomethacin obtained by the authors at pH 5 and 7. The concentrations reached constant values after 2 days in most cases. However, when the solid amount added ($C_t$) was very low (40 μg/mL) at pH 5, a period of five days was required to attain the constant solubility value. As indicated in Figure 3, the apparent equilibrium solubility was affected by the amount of solid added to the dissolution medium. The increase in the solid amount led to a decrease in the apparent solubility at pH 5 (Figure 3a), whereas opposite tendency was observed at pH 7 (Figure 3b). The buffer concentration and thus buffer capacity was not reported by the authors. The pH values at equilibrium of the solubility samples were not measured, and therefore the impact of ionization on the indomethacin (pKa 4.1)[68] solubility cannot be ascertained. The observed effect of decrease in solubility with excess indomethacin at pH 5 could be due to suppression of ionization of indomethacin. The observed effect of excess solid on indomethacin solubility at pH 7 is not interpretable without analysis of the solid form at equilibrium and pH at equilibrium. This effect could possibly be due to either self-association

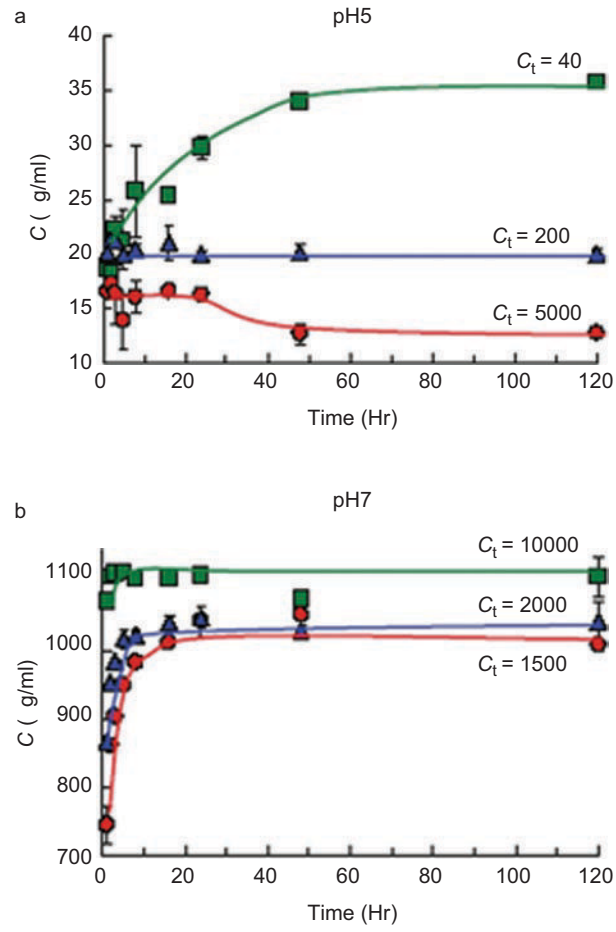


**Figure 3.** Time dependence of indomethacin (IDM) concentration, C, during dissolution at (a) pH 5 and (b) pH 7. Ct is the solid amount added (μg/mL). All measurements were done in triplicate. The standard deviations are shown in error bars, although most of the bars are hidden in the symbols. Figure adapted from reference [26]. (Reproduced with permission from Pharmaceutical Research, Springer Netherlands).

or partial formation of sodium salt of indomethacin or potentially the formation of co-crystals. The formation of dimers has been reported for carboxylic acids and their salt forms.[69,70] In any case, these examples highlight the importance of taking into account physico-chemical factors during the set-up of experimental procedures and analysis of solid at equilibrium during the measurement of solubility. In contrast, the amount of excess solid on the measured solubility using a shake-flask method was examined by Baka et al.[71] for hydrochlorothiazide. In this case, there were no significant differences in solubility values observed when different excess amounts of hydrochlorothiazide were used. Clearly, the impact of excess solid on the measured solubility is complex, and at the very least, the amount per unit volume used in the study needs to be specified.

Elamin et al.[27] and Mosharraf et al.[34] have suggested that if the particle surface is disordered to some degree, the apparent solubility will be a function of the amount of

solid dispersed in the medium during the attempted solubility measurement. It must be acknowledged, however, that any mixture of disordered and crystalline phases that is homogeneously distributed throughout the particle, where the disordered phase has at least partial access to the surface, would yield the same result. In such cases, the concentration of the dispersion of the powder in the medium determines the amount of disordered solid available for dissolution. Under such circumstances, the saturation concentration would increase as the amount of drug added is increased, until finally at higher additions the saturation concentration reaches a plateau measuring the solubility of the (partially) disordered phase, not the solubility of the crystalline phase. This would be true only when the time for crystallization of the supersaturated solution onto the existing crystalline phase is much longer than the time for the solubility measurement. Elamin et al.[27] measured apparent solubilities of different forms of griseofulvin (unmilled and milled) by adding different amounts of the test materials, ranging from 60 and 960 mg/L, to purified water. The solubility values of the unmilled (presumably, highly crystalline) griseofulvin were essentially the same regardless of the amount of material added. This is to be expected for a 100% crystalline material. However, as expected, the milled griseofulvin showed a proportional increase in the initial peak concentrations (what we propose to call 'apparent solubility' values) when increasing amounts were added to the dissolution medium, until it reached the level characteristic of the 100% amorphous solid prepared by quenching from a melt. Clearly, the disordered phase produced by milling had an apparent solubility essentially that of the melt-quenched amorphous solid because the crystallization of griseofulvin is slow relative to the period of measurement of solubility.

Mosharraf and Nystrom also investigated the solubility of partially crystalline systems.[72] These authors reported the minimum dispersion concentration required in the corresponding suspension in order to reach the saturation concentration plateau characteristic of the 100% amorphous phase. The greater the degree of disorder, the lower is the amount required. In this study, the 100% amorphous samples were prepared by melt-quenching. The authors concluded that, in partially crystalline systems, the saturation concentration is a measure of the 'solubility' of the disordered region at the surface.

## Issues with complex solid systems

In this section we discuss measurement issues pertaining to special complex systems such as amorphous drug, amorphous drug with a carrier, and amorphous drug in a dosage form.

### *Defining amorphous drug solubility*

There are limited numbers of literature reports focused on measurement of solubility of an amorphous drug alone (i.e. without carrier). In one study the solubility of amorphous forms of thiazide diuretics prepared by spray-drying were determined at 37°C in 0.1 N HCl containing 1% PVP to inhibit crystallization.[19] The increase in apparent solubility (i.e. maximum concentration during dissolution) of the amorphous phase relative to the corresponding crystalline solid ranged from nearly 10-fold in the case of polythiazide to 1.1-fold for hydrochlorothiazide. Of course, since the amorphous form has a high free energy, the solubility is expected to be substantially higher than that of the crystalline form; in fact, the low apparent solubility enhancement ratio of 1.1 is likely due to crystallization during the measurement. In another study, Gupta et al.[73] measured the aqueous solubility of amorphous celecoxib in water and attempted to theoretically predict the solubility advantage of the amorphous form from measured thermal properties. While the aqueous apparent solubility of amorphous celecoxib was found experimentally to be higher than its crystalline counterpart, the measured values were much less than the theoretically predicted values, likely in part due to crystallization during the experimental measurement process. A similar procedure was also used by Chawla and Bansal[74] for apparent solubility measurement of amorphous irbesartan in different media. These literature studies emphasize the difficulties encountered in accurately measuring amorphous drug solubility due to crystallization of the amorphous drug during the attempted experimental measurement. The use of separate terms for peak concentrations and sustained concentrations during solubility measurements will clarify the nature of the values being reported. As discussed previously, consistent use of the terms, 'apparent solubility' and 'apparent equilibrium solubility' may provide a useful distinction.

### *Amorphous drug with a carrier*

The majority of the literature on amorphous drug dissolution includes a carrier to form a solid dispersion. The system may be totally amorphous with the drug molecularly dispersed in the amorphous carrier, but particularly in some of the older literature, the drug may well have been micro-crystalline.[75–77] This type of system is widely applied to enhance the apparent solubility and bioavailability of poorly soluble drugs.[78–81] Solid dispersions with various polymers such as polyvinylpyrrolidone (PVP),[82] hydroxypropylmethylcellulose (HPMC),[83,84] hydroxypropylcellulose and silicates such as calcium silicate, silicon dioxide, magnesium aluminosilicate (Neusilin®),[85,86] cyclodextrins,[87] and lipids,[82]

among others have been reported. These solid dispersions were obtained by using various techniques such as melt quenching, solvent evaporation, spray drying, and milling.

In most modern applications, a solid dispersion is intended to be an amorphous dispersion of one or more active ingredients in an inert carrier in the solid state. However, even a dispersion of micro-crystalline drug in an amorphous carrier might be expected to produce a higher dissolution rate simply because such a system would yield a dispersion of small (high specific surface area) crystals in the dissolution medium. If the solid dispersion is indeed a molecular dispersion of the drug in the amorphous carrier, a very high transient concentration, higher than the solubility of the amorphous state, may result. That is, rapid dissolution of the highly soluble matrix will produce (briefly) a solution of drug in the dissolution medium at a concentration given by the total amount of drug divided by the volume of the dissolution medium, at least until phase separation occurs. Phase separation might then produce a colloidal dispersion of amorphous drug phase in the aqueous dissolution medium, which will likely crystallize, quickly or slowly depending on the system. The time required to nucleate the amorphous drug phase is likely short, but these phenomena have not been systematically studied. In addition solubility of the drug can be increased by the excipients used in solid dispersion preparation.[88,89] It is useful to measure the solubility of a crystalline form of the drug in pre-dissolved excipient to determine the effect of the excipient on drug in the solution state, before attributing increases in solubility to the amorphous form of the drug in the solid dispersion.

## Summary and conclusions

A large number of methods have been used to assess the solubility of crystalline, partially crystalline and amorphous solids. Even though high-throughput assays have recently become available, the generation of accurate solubility data remains a relatively expensive and time consuming activity. Among the different methods available, solubility methods that are based on precipitation from solutions prepared from DMSO (or other organic solvent) stock after addition to water are particularly problematic due to the potential for lack of equilibrium and the failure to identify the solid whose apparent solubility is being measured. Classical shake-flask methods, though time-consuming and labor intensive, remain the most suitable method for the measurement of thermodynamic (equilibrium) solubility.

In recent years there has been increased interest in the study of amorphous forms of pharmaceuticals because of their enhanced solubility and potentially greater bioavailability relative to their crystalline counterparts. Here, we have attempted to critically review the experimental methods used to measure solubility of both amorphous drug and dispersions of drug in amorphous carriers. It is well known that accurate measurement of amorphous drug solubility is often difficult or impossible due to crystallization that can occur during experimental measurement of solubility.[90,91] In the appendices (online version only) we have provided recommendations of preferred methods for the determination of aqueous solubility of crystalline and amorphous drug based on current knowledge.

## Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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## Appendix

### *Recommended procedures for solubility measurement*

**A. Crystalline drug**

Here we suggest a material sparing methodology by which one may measure the thermodynamic solubility of a crystalline drug. In general, this methodology will measure the solubility of the thermodynamically most stable form. Steps 1–10 describe a thorough process that has a considerable history of successful use in industry. However, we acknowledge that variations of this procedure may serve equally well in many practical applications.

Temperature cycling method for measurement of thermodynamic solubility:

1. Weigh out a known amount (5–20 mg) of compound.
2. Add 1–2 mL of vehicle of interest (e.g. water).
3. Vortex the sample.
4. Observe if the compound dissolves completely. If the compound is in solution, additional compound should be added before experiment is begun (a known amount, 5–15 mg excess drug is desired).
5. Once suspensions of crystals in aqueous medium have been obtained, place the samples in the temperature controlled bath. The samples are placed on a shaker (for example, LabQuake, Lab Industries,

Inc. Berkeley, CA, USA) to ensure mixing via rotation through the entire cycle. Cycle temperature used as per the following method:*

(1) 25°C for 1 min;
(2) 40°C for 8 h;
(3) 15°C for 5 h;
(4) 25°C for 12 h.

6. Observe samples after temperature cycling. If there is no solid material left in the tube, add more solid material and repeat temperature cycling.
7. If there is solid material left in the tube, centrifuge the sample. Collect the supernatant, measure its pH, and analyze by HPLC for drug concentration.
8. Collect solid material. Analyze the wet solid material by X-ray powder diffraction (XRPD) and examine the diffraction spectrum for any changes relative to the control sample.
9. Dry solid material under vacuum overnight at 40°C (depending on the potential for chemical instability; the solid material can also be dried under vacuum at room temperature).
10. Analyze the dry material by DSC (against control samples) for change in melting point.
11. Run the following solubility procedure at 25°C if significant degradation is observed after temperature cycling:
    (a) Add 5–20 mg of solid powder in a suitable size glass vial, and add 1–2 mL of aqueous vehicle (e.g. water).
    (b) Briefly vortex mix for 30 s to ensure adequate dispersion of solid.
    (c) Rotate vials containing excess drug in aqueous media on a vial shaker for a period of 48 h in the temperature controlled bath at 25°C.
    (d) Take two time points: 24 and 48 h to ensure that the equilibration has been reached.
    (e) After 24 h of equilibration, record pH of slurries and either centrifuge or filter aliquots of slurries and assay for drug content by HPLC.
    (f) Repeat above 24 h later. If the values at 24 h and 48 h are within ± 10% then assume equilibrium has established.

**B. Amorphous drug**

Based on work in our laboratory we recommend the following procedure for measuring solubility of amorphous solids. If crystallization occurs during the measurement of experimental solubility, the solubility obtained is 'apparent solubility' when crystallization occurs rapidly and 'apparent equilibrium solubility' when crystallization occurs beyond the clinically relevant time-scale. In the case of an 'apparent solubility', the value will be less, often much less than the equilibrium solubility of the amorphous phase.

1. Screen the amorphous drug powder through a 100-mesh screen (150 μ size opening) and select that retained on a 200-mesh screen (75 μ size opening) in order to avoid fines that dissolve faster and larger particles that might cause difficulties in dispersing the drug in water, thereby improving reproducibility of the concentration:time profile measurements.
2. Add a known amount of excess amorphous drug (at least 10× higher amount than corresponding crystalline drug solubility) powder to a dissolution vessel in a 250 mL dissolution medium in a Type II USP apparatus at 25°C.
3. In order to improve the powder dispersion, use paddle speed of at least 300 rpm.
4. Draw samples at different time points and filter using an appropriate filter.
5. Dilute samples immediately in organic solvent to prevent precipitation and/or crystallization of drug.
6. Assay by HPLC for drug concentration.
7. Pull an additional sample of unfiltered solid at each time point and analyze by polarized light microscope (PLM) for appearance of crystallinity.
8. Generate the concentration-time dissolution profile, and note the time at which crystallinity is first detected.

*Note: Increasing the temperature to 40°C will increase the drug concentration in aqueous medium providing higher degree of supersaturation relative to 25°C to facilitate the nucleation at 25°C or lower temperature. Lowering the temperature to 15°C from 40°C will lower the drug solubility, further increase the degree of supersaturation and promote nucleation of the thermodynamically stable form. Raising the temperature of the sample back to 25°C allows equilibration of thermodynamically stable form to the temperature at which solubility measurement is intended.

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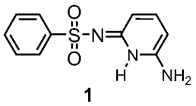
Crystal Polymorphism

# Unmasking a Third Polymorph of a Benchmark Crystal-Structure-Prediction Compound**

*Saikat Roy and Adam J. Matzger**

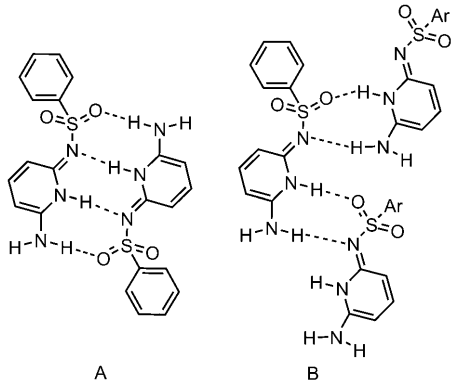
The ability of a chemical substance to adopt more than one crystal structure is known as polymorphism,[1] a phenomenon which can be both beneficial and problematic.[2] Serendipity often plays a key role in the discovery of new forms, because no general methodology exists for producing new forms of a given compound. Understanding the origin of polymorphism and controlling the outcome of crystallization processes to avoid undesired forms is therefore a current goal.[3] A closely related challenge is the prediction of a crystal structure from a given chemical structure. This process, which involves generating and energetically ranking many hypothetical polymorphs, is referred to as crystal-structure prediction (CSP).[4] The importance of CSP has been recognized through the "blind tests" conducted by the Cambridge Crystallographic Data Center (CCDC). However, even simple rigid molecules in common space groups present a considerable challenge,[5,6] and prediction of flexible molecule crystal structures is even more difficult owing to the much larger parameter space to be explored.[7,8] Examination of kinetic and thermodynamic issues in crystallization can provide insight into the mechanism of nucleation and aid development of better methodologies for CSP. In this spirit, we undertook the study of 6-amino-2-phenylsulfonylimino-1,2-dihydropyridine (**1**, Scheme 1), which was part of the second CCDC blind test in 2001 (molecule VI)[5b] and then subjected to additional scrutiny after the contest.[9]



**1**

***Scheme 1.*** Chemical structure of blind-test molecule **1**.

The dihydropyridine **1** prediction exercise ended with disappointment, as none of the participants of the blind test were able to predict the crystal structure correctly.[5b] Further study[9,10] yielded a second polymorph of molecule **1**, claimed to be the thermodynamic form; form II contained dimer hydrogen-bonding four-point synthon A (Scheme 2). It was proposed[9] that because form I contains two-point synthon B, it facilitates 1D growth and that this polymorph was the kinetically favored form. This rationale neatly explained why thermodynamic predictions of polymorph stability employed in most CSP approaches failed to find this form and implicated the initial crystallization conditions in leading to an unstable form.



A B

***Scheme 2.*** The four-point synthon A and two-point synthon B.

Because polymorphism is so common in sulfonamides,[11] we were encouraged to reopen the investigation of molecule **1** utilizing functionalized cross-linked polymers as heteronuclei to reveal new regions of polymorph space.[3a] Polymer-induced heteronuleation (PIHn) is a strategy that has proven effective for a wide range of compounds.[12] Crystals of molecule **1** were grown from ethanol using PIHn (see the Supporting Information). Three types of crystal morphologies were observed: block-like crystals of form I, needle-type crystals of form II, and hexagonal plates (Figure S1 in the Supporting Information). Raman spectroscopic analysis of these crystals revealed three distinct forms with characteristic Raman spectra (Figure S2 in the Supporting Information), and the hexagonal plates where denoted as form III. The powder X-ray diffraction (PXRD) patterns of these three forms are distinct (Figure 1), and block-like form I and needle-like form II closely match predicted patterns based on the previously reported forms of molecule **1**.[9] However, form III, although distinct (Table S2 in the Supporting Information), has some similarities in PXRD profile with form II, and this finding necessitated further study by single-crystal X-ray diffraction.

The earlier reported structures[9] of form I and form II revealed the presence of synthon B and synthon A (Scheme 2) as building blocks of the crystal lattices with one and two molecules in the asymmetric unit, respectively. Form III is orthorhombic[13] with one molecule in the asymmetric unit. Moreover, form III exhibits two-point synthon B, similar to form I (Figure 2c). Form I and form III have a similar catemer synthon with N–H···N hydrogen bonds

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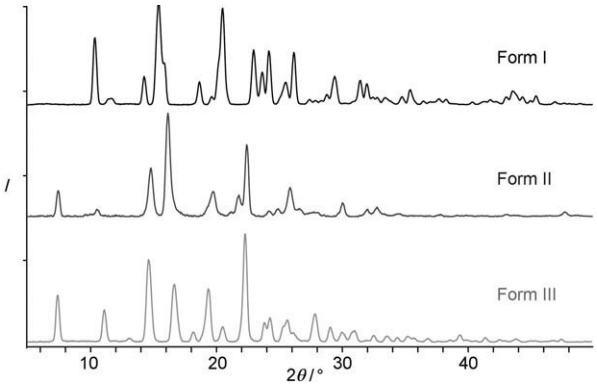


***Figure 1.*** Experimental powder X-ray diffraction pattern of form I, form II, and form III.

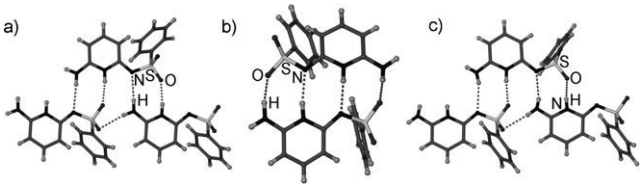


***Figure 2.*** Hydrogen-bonding synthons in a) form I (synthon B), b) form II (synthon A), and c) form III (synthon B).

of an amine with an imine N atom and N–H···O hydrogen bonds between secondary ring amine and the O atom of a sulfonyl moiety. Both form I and form III possess N–H···O interactions between translationally related molecules along the *b* axis. However, form II is quite different structurally, with N–H···N and N–H···O hydrogen bonding between two symmetry-independent molecules to make a dimer (Figure 2b). In form II, these 0D dimers are connected by N–H···O hydrogen bonds to make 2D sheet arrangements. Although form I and form III share the same synthon in their structures, orientations of phenyl rings are different, resulting in completely different packing (Figure 3a,c). The phenyl groups of the molecule in the dimer of form II are also in a *syn* orientation, as in form III, which leads to similar stacks of layered structures in both the forms (Figure 3b,c). Conformational analysis of the four symmetry-independent molecules in the three forms of molecule **1** reveals that they each have distinct molecular conformations (Figure 4). This conformational polymorphism arises from the flexibility along C-C-S-N, C-S-N-C, and S-N-C-N torsion angles.

It was previously proposed[9] that synthon A in form II is more preferred because of its shorter and more linear hydrogen bonding. Moreover, a lattice energy calculation (CVFF95) on the crystal structures suggested that form II is more stable by 0.86 kcal mol$^{-1}$. The Hirshfeld surfaces[14] of the polymorphs of molecule **1** (Figure 5) reveal that form I has a greater contribution from O···H (26.1%) and lesser N···H bonding (8.2%) than form II (21.5% and 10.4%, respectively). In form III, the contribution of O···H hydrogen bonds is less (21.1%), because it does not contain C–H···O interactions, unlike form I. Overall, the analysis suggests that form II has a higher contribution from N···H bonds than form I and form III. Hirshfeld surface fingerprint plots (Figure S4 in the Supporting Information) show the presence of close O···H contacts in form I and close N···H interactions in form II.

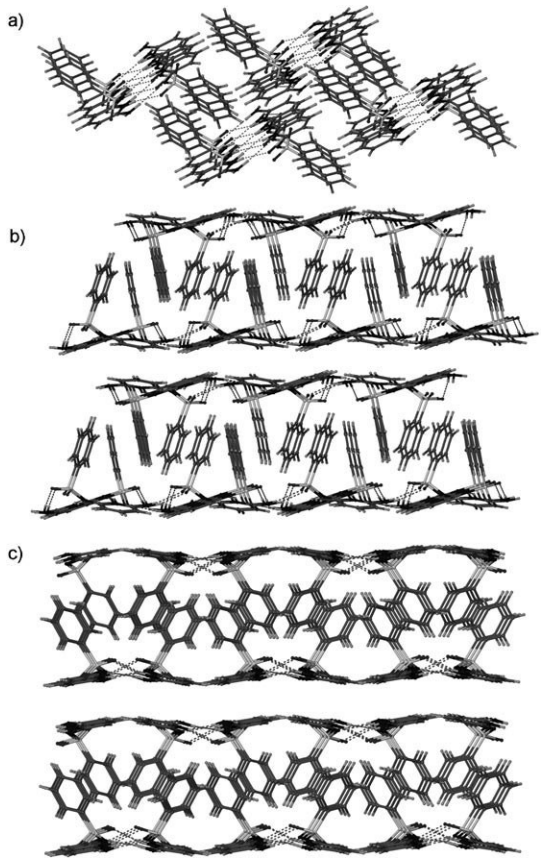


***Figure 3.*** a) *Anti* arrangement of phenyl rings in catemers of form I. *Syn* orientation of phenyl rings in stacked layers of b) dimer in form II and c) catemer of form III.

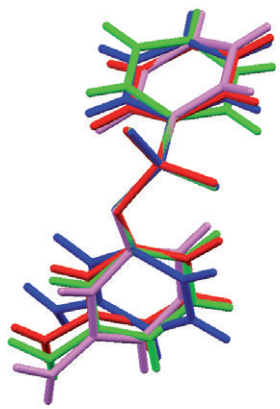

***Figure 4.*** Overlay of symmetry-independent molecules of form I (pink), form II molecule A (green), form II molecule B (blue), and form III (red).

Thermomicroscopy and differential scanning calorimetry (DSC) experiments confirm that form I and form III have approximately 12 °C higher melting point than form II

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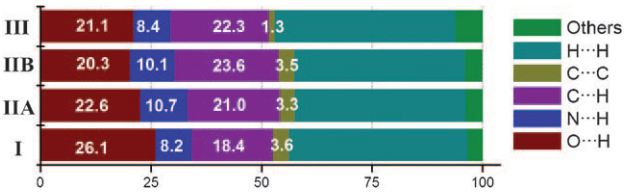


***Figure 5.*** Relative contribution to the Hirshfeld surface for the various intramolecular contacts for the molecules in form I, form II molecule A, form II molecule B, and form III.

(Figure S5 in the Supporting Information). Moreover, it is observed that form II has a lower enthalpy of melting ($\Delta H = 9.56$ kcal mol$^{-1}$) than form I ($\Delta H = 11.81$ kcal mol$^{-1}$) and form III ($\Delta H = 10.92$ kcal mol$^{-1}$). The higher enthalpy of form I indicates more favorable packing energy than for form II and form III (Figure 3).

Previous lattice-energy calculations[9] predicted that form II is more stable by about 1 kcal mol$^{-1}$ than form I and, along with synthon energy computational results, concluded that form II is thermodynamically favored, while form I is kinetically favored. Our experimental results led us to reexamine this issue with a computational procedure based on the COMPASS force field that has been demonstrated to agree well with experiment for a range of polymorphic pharmaceuticals.[15] However, this method incorrectly predicts that form II is the lowest-energy arrangement, thus highlighting the challenge of applying computational approaches to this molecule.

Enthalpic measurements alone cannot predict which form is most stable at room temperature. To determine the relative free energy relationships, solvent-assisted polymorphic transformation experiments were carried out by combining polymorphs pairwise in ethanol; both form II and form III convert to form I, thus demonstrating that the crystal structure originally reported as the blind-test solution is the thermodynamically most favorable one at room temperature. To quantify these differences, relative free energies were determined (see the Supporting Information).[12d] Form I is the most stable at 299 K and is more stable than form II by 0.44 kcal mol$^{-1}$ and than form III by 0.23 kcal mol$^{-1}$. These experiments demonstrate that form I is the thermodynamically favored form and that form II is least stable among the three polymorphs. In this context, the results of all teams in the 2001 blind prediction trial were reexamined. The two most stable forms, forms I and III, were absent from all submitted structure predictions.

In conclusion the thermochemical and physiochemical relationship among the polymorphs of a conformationally flexible blind-test molecule has been established based on experimental findings. Moreover, a third novel form has been found for this important benchmark compound. These results demonstrate that though form I is the thermodynamic form, it was beyond prediction; this result highlights the importance in improvements of computational methodology for CSP. Finally, 20 years ago Maddox commented that the lack of success in CSP is a "continuing scandal" and noted that the "X-ray crystallographer need not worry—yet".[16] After two decades, although improvements have been made, there is still considerable job security for the experimental chemist and crystallographer.

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**Keywords:** crystal growth · crystal-structure prediction · Raman spectroscopy · solid-state structures · X-ray diffraction

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# Dealing with the Impact of Ritonavir Polymorphs on the Late Stages of Bulk Drug Process Development

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**Abstract:**

**Ritonavir (Kempf, D. J.; Marsh, K. C., Denissen, J. F.; McDonald, E.; Vasavanonda, S.; Flentge, C. A.; Green, B. E.; Fino, L.; Park, C. H.; Kong, X. P.; Wideburg, N. E.; Saldivar, A.; Ruitz, L.; Kati, W. M.; Sham, H. L.; Robins, T.; Stewart, K. D.; Hsu, A.; Plattner, J. J.; Leonard, J. M.; Norbeck, D. W. *Proc. Natl. Acad. Sci. U.S.A.* 1995, *92*, 2484) is Abbott's novel protease inhibitor, for human immunodeficiency virus (HIV), the causative organism of acquired immunodeficiency syndrome (AIDS). It is marketed as Norvir. From the discovery of ritonavir until the new drug application (NDA) filing, only one crystalline form was known to exist. Attempts to identify other possible crystal forms were unsuccessful. Two years after the launch of Norvir to the market, some lots of Norvir capsules failed a dissolution specification. Investigation of this phenomena revealed the existence of a crystal form of ritonavir other than the one already known (Form I). This new crystal form was designated as Form II. The two crystal forms are polymorphs and differ substantially in their physical properties such as solubility. In this article, we will discuss the challenges these polymorphs created for the bulk drug substance as well as for the formulation, and how we dealt with these challenges.**

## Introduction

Ritonavir was discovered at Abbott Laboratories in late 1992. The new drug application (NDA) was filed in December, 1995. Commercial start-up followed in January, 1996, and FDA approval, in March, 1996. The final drug product, Norvir, was introduced to the market as a semisolid capsule formulation and as a liquid formulation. In early 1998, an unexpected occurrence was observed. Many final product lots started failing the dissolution test. A large portion of the drug substance was precipitating out of the final (semisolid) formulated product. Further investigation into this matter revealed the fact that a new, previously unknown, thermodynamically more stable and much less soluble crystalline form had emerged. This new polymorph was referred to as Form II of ritonavir, and the originally known solid form was referred to as Form I. The semisolid formulation consisted of a nearly saturated solution of Form I. Since Form II was much less soluble in the solvents used for formulation, it was very supersaturated with respect to Form II. Soon, Form II started spreading, and all attempts to formulate the semisolid capsules failed. The drug substance was converted to Form II and precipitating out of solution. This put Abbott into a market crisis. The supply of the semisolid capsules was depleting very quickly. Once Form II was found in the formulation, samples were brought into our laboratories to study the properties of this form. Within a few days all of the lots of ritonavir prepared in the lab turned out to be Form II. From that point on all attempts to make Form I failed until a process was developed to control Forms I and II. A team of scientists who had been exposed to Form II visited our manufacturing facility in Italy to investigate if any significant changes had been made to the bulk manufacturing process. Until this time no detectable quantities of Form II had been detected in the bulk drug lots. Whether or not it was coincidence, soon after this visit significant amounts of Form II started showing up in Abbott Italy bulk drug during the manufacturing process. Although the origin of Form II remains highly debatable, the fact was that this issue had to be addressed as soon as possible. A two-prong approach was taken to tackle this problem. First was development of a formulation to accommodate Form II of ritonavir. Second was the establishment of a controlled process to consistently generate Form I bulk drug. It was also feared that any contamination of Form II in the bulk drug will not go completely into solution and hence can later serve as an initiator of Form II crystallization in the formulated drug.

The first synthetic route used for commercial production of ritonavir is referred to as the phase I process.[2,3] Subsequent development with very minor changes lead to the phase II process. In both cases, the drug substance is synthesized by a convergent coupling of three key intermediates in a series of chemical reactions called the assembly steps, as shown in the Scheme 1. In the phase I synthetic process, the three key intermediates are boc-core-succinate, 5-wing, and 2,4-

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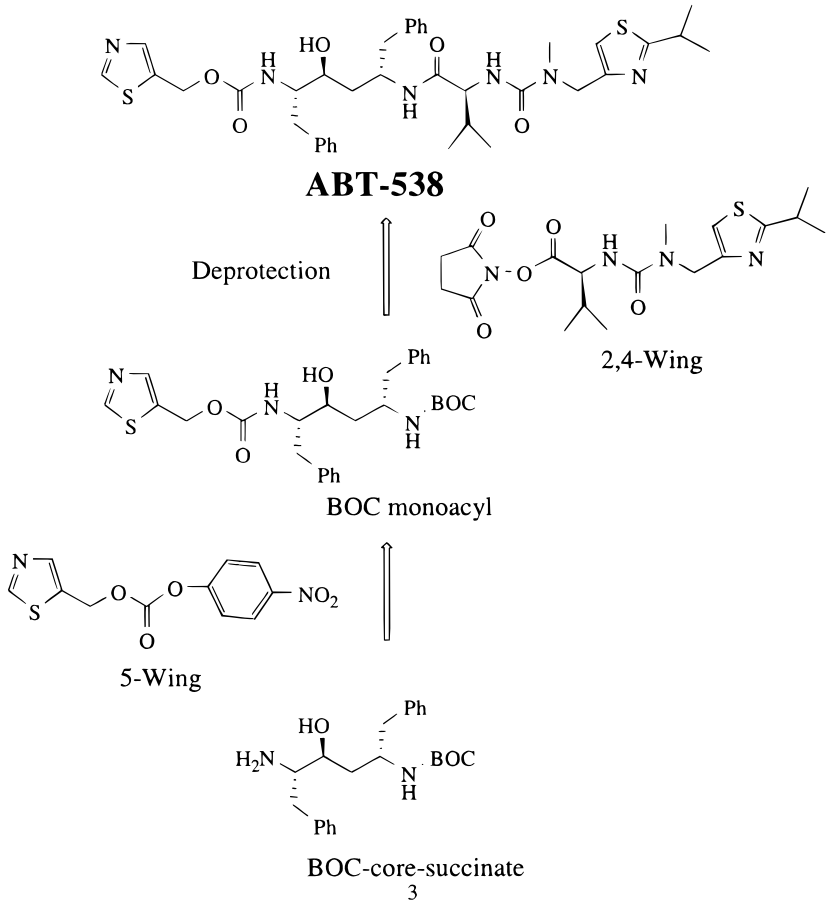
*Scheme 1*

**Synthetic route:**




wing acid. The 2,4-wing acid was converted by in situ reaction to the 2,4-wing active ester, which underwent further assembly reaction to produce ritonavir. The phase II synthetic process differs from the phase I process as it uses 2,4-wing active ester as a starting material for the convergent synthesis of ritonavir.

## Polymorphism

Polymorphism is the ability of a solid compound to exist in more than one crystalline form. These crystalline forms, although chemically identical, result from a different ordered arrangement of molecules within the crystalline lattice. Consequently, two polymorphs of the same compound can differ in physical properties that depend on the crystal lattice stability, such as melting point and solubility. The solubilities of different polymorphs of the same compound reflect the differences in free energy between their respective crystalline states (which are different for each polymorph) and the solvated state. Thus, at a given temperature, different polymorphs may have significantly different solubility values. It is widely recognized that once a compound is in solution, any differences in solid-state structure are no longer applicable.[4] This is indicated by the decision process regarding polymorphism in the Proceedings of the Fourth International Conference on Harmonization, Brussels, 1997, "for a drug product that is a solution, there is little scientific rationale for polymorph control".[5] Once the solid has been completely dissolved and there are no undissolved crystals present, the properties of the compound are unaffected by the original crystal form.

Since the discovery of ritonavir Form II, several characterization studies have been conducted. Forms I and II have different crystal habits. When examined using polarized light microscopy, Form I is usually observed as lath crystals or rods, whereas Form II crystals appear as fine needles.

In Form II, all of the strong hydrogen bond donors and acceptors have been satisfied, and the hydrogen bonds are strong. The difference between the solubilities of Forms I and II can be explained in terms of the strength of the hydrogen bonds present within the crystal. Since the strength and completeness of the hydrogen bonding has attained the maximum possible in the Form II lattice, it is not thought possible that another undiscovered polymorph of ritonavir would exist with equivalent or lower solubility than that of Form II.

Additional studies have been carried out to investigate the crystallization behavior using different solvent systems

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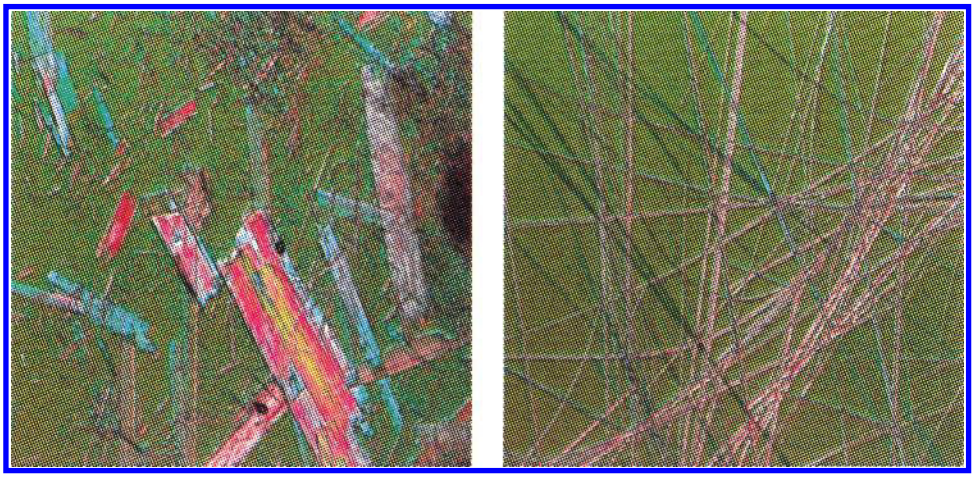

***Figure 1.*** **Video micrograph of crystal Form I (left) and Form II (right).**

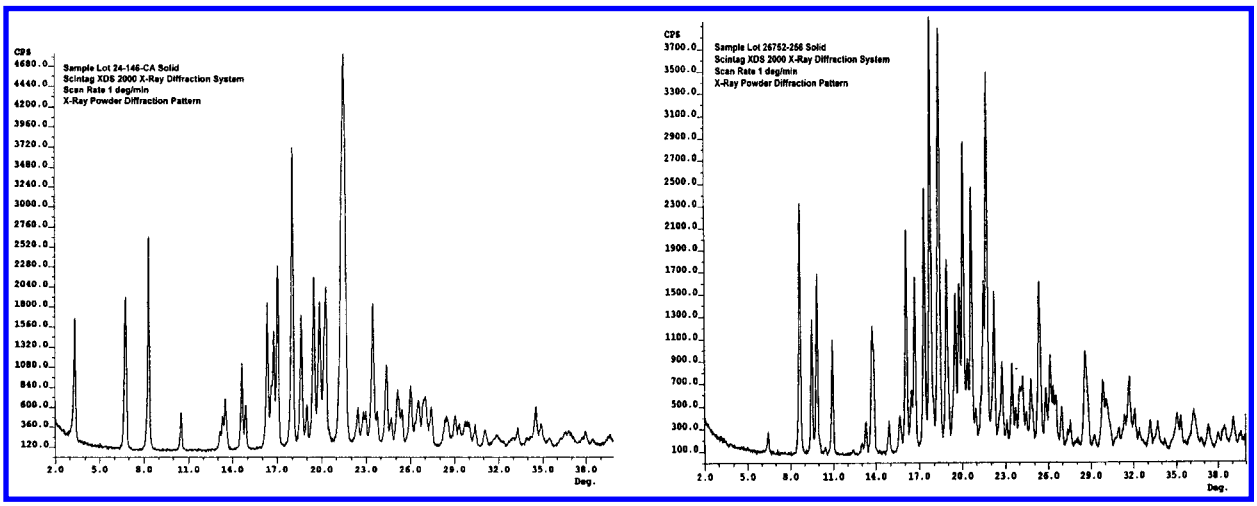


***Figure 2.*** **X-ray powder diffraction of Form I (left) and Form II (right).**

and different crystallization techniques. Results from these studies indicated that ritonavir (Form I or Form II) recrystallized predominantly as Form I or amorphous material. These findings are consistent with the Ostwaldt rule which states that the less stable form precipitates first and crystallizes more easily and preferably.

Solid-state characterization by means of $^{1}$H and $^{13}$C nuclear magnetic resonance (NMR) spectroscopy as well as NIR (near infrared spectroscopy) and mid-IR confirmed that ritonavir can exist in two polymorphic forms. Similar spectroscopic studies using both NMR and IR on solutions of both Forms I and II ritonavir confirm that once in solution the ritonavir is identical regardless of the original polymorph dissolved. Solutions prepared using Form I and solutions prepared using Form II gave identical NMR and IR spectra.

Therefore, Form I or Form II ritonavir drug substance are both considered suitable for use in production of Norvir soft gelatin capsules as long as the manufacturing conditions ensure complete dissolution of the drug.

## Form I and Form II Characterization and Analytical Information

The Form II crystals were analyzed by using several established techniques for characterizing polymorphic forms. The results of the comparison of Forms I and II are detailed below.

**Optical Microscopy.** As shown in Figure 1 the crystal habits of Forms I and II differ when examined using a polarized light microscope. Form I is usually observed as lath crystals or rods, while form II is almost always fine needles.

As shown in Figure 2, the two polymorphic forms have very different and distinctive patterns. Form I has a characteristic combination of peaks at 3.32 and 6.75 $2\theta$, while Form II is missing these peaks and has characteristic peaks at 9.51, 9.88, and 22.2 $2\theta$.

**Solid-State Near Infrared Spectroscopy.** The primary peaks for Forms I and II differ by approximately 19 wavenumbers: 6085.6 wavenumbers (1.653 $\mu$m) for Form I and 6066.7 wavenumbers (1.648 $\mu$m) for Form II.

**Solid-State $^{13}$C Nuclear Magnetic Resonance (NMR).** The solid state NMR spectra for the two forms differ significantly in the ranges of 150−180 ppm and 190−215 ppm.

**Melting Points.** There is an approximately 2−3 °C higher melting point for Form II (approximately 125 °C) versus Form I (approximately 122 °C). The heat of fusion for Form II (87.8 J/g) is greater than for Form I (78.2 J/g).

## Forms I and II Solubilities

A comparison of the solubility profiles of the two forms in a series of ethanol:water solvent mixtures at 5 °C is

***Table 1.*** **Solubility profile in various hydroalcoholic solvent systems at 5 °C**

| ethanol/ water | 100/1 (mg/mL) | 95/5 | 90/10 | 85/15 | 80/20 | 75/25 |
|---|---|---|---|---|---|---|
| Form I | 90 | 188 | 234 | 294 | 236 | 170 |
| Form II | 19 | 41 | 60 | 61 | 45 | 30 |

***Table 2.*** **Ritonavir polymorph I and II solubility**

| solvent | ratio | mg/mL (Form I) | mg/mL (Form II) |
|---|---|---|---|
| Temperature = 70 °C | | | |
| ethyl acetate | NA | 1250 | 825 |
| ethyl acetate:heptanes | 2:1 | 266 | 125 |
| ethyl acetate:heptanes | 1:1 | 62.5 | 31 |
| ethyl acetate:heptanes | 1:2 | 11 | 6 |
| Temperature = 50 °C | | | |
| ethyl acetate | NA | ND[a] | 26.85 |
| ethyl acetate:heptanes | 2:1 | 9.26 | 6.67 |
| ethyl acetate:heptanes | 1:1 | ND | 2.38 |
| ethyl acetate:heptanes | 1:2 | ND | 0.51 |
| Temperature = 25 °C | | | |
| ethyl acetate | NA | 14.87 | 5.45 |
| ethyl acetate:heptanes | 2:1 | 4.43 | 1.85 |
| ethyl acetate:heptanes | 1:1 | ND | 0.66 |
| ethyl acetate:heptanes | 1:2 | 0.33 | 0.21 |

[a] ND = Not Determined.

presented in Table 1. As shown by these data the solubility profiles parallel each other, with Form II having significantly lower solubility throughout the series.

The solubilities of ritonavir polymorphs I and II in the bulk drug manufacturing process crystallization system are shown in Table 2. The solubility of polymorph I is significantly higher than that of polymorph II.

## Evaluation of Bulk Drug Manufacturing for a Correlation to Form II Found in the Semisolid Formulation

After Form II was found in the semisolid formulation, an investigation was done to correlate manufacturing changes and the presence of Form II. The only significant process change implemented in manufacturing was during the final washing as described below.

Normal wash sequence was the following: aqueous potassium carbonate, aqueous citric acid, aqueous potassium carbonate, aqueous citric acid, and water followed by crystallization.

Modified wash sequence was the following: aqueous potassium carbonate, aqueous citric acid, aqueous potassium carbonate, aqueous citric acid, dilute aqueous potassium carbonate, and water followed by crystallization.

The modified wash sequence was implemented to minimize early eluting impurities in the bulk drug. After the modification, at least 12 lots were used in the formulation without any failure for dissolution; therefore, this modification could not be directly responsible for the generation of Form II. Graphs were plotted to find the correlation of Form II appearance with bulk drug potency, total impurities, individual impurities, largest unknown impurities, pH of the bulk drug, and amorphous content. All of the studies indicated no correlation between these factors and the presence of Form II.

**Impact of Form II on the Manufacturing of Bulk Drug.** Manufacturing lots that generated Form II during final crystallization had a 50% failure rate. Failures were mainly for three reasons: solvent front impurities, residual solvents, and ethyl carbamate impurity. Extended drying time was needed to remove the residual solvents. It was found that early eluting impurities could be removed by an additional potassium carbonate wash prior to final crystallization. The carbamate impurity was linked to the source of the starting material (5-wing HCl containing the precursor impurity ethyl-*p*-nitrophenyl carbonate). This impurity was always formed whenever the source contained the precursor impurity. If the final product crystallized out as Form I, the carbamate impurity was washed away with the mother liquors; on the other hand, it cocrystallized with Form II. Thus, this impurity could be eliminated either by controlling the impurity profile of the starting material (5-wing HCl) or by controlling the final crystallization process to give Form I. In conclusion, even though a new formulation was developed to accommodate Form II solubility and no specification on the crystalline form of the bulk drug was required, it was still desirable from the manufacturing point of view to target Form I as the final crystal form. This also reduced processing time during formulation since Form I dissolves much faster than Form II.

**Back to the lab.** First, we decided to address memory retention of Form II in solution form. We found that, although Form II was much less soluble than Form I, it could be sonicated to form a highly supersaturated solution with respect to Form II and this solution could be maintained under a closed system to prevent any external contamination of Form II. This solution was then seeded with Form I to cause crystallization. The powder X-ray results showed only Form I as the product. This clearly suggested that the crystal form memory was not retained in the solution (Note: it is known that in the case of ritonavir if there is any contamination of Form II, the product is always Form II even if it is seeded with Form I).

Encouraged by this observation we moved on to pursue the process to selectively generate Form I. Super seeding is a common approach used to achieve the formation of the less thermodynamically stable, yet desired, polymorph. As high as 50% seeding was considered for this purpose. By adding such a high amount of seed the throughput was reduced by 50% which was a huge drawback. We developed a very interesting idea to simulate super seeding without actually using large amounts of seeds, by using a reverse addition technique. The reverse addition technique was as follows: a small amount of seeds was stirred in the required amount of antisolvent. To this, the solution of product, in a crystallizing solvent, was slowly added. Since a very small amount of solution was added to the small amount of seeds originally present, this created the same effect as superseeding, and as the addition progressed, the product that

*Table 3*

| ethyl acetate | heptanes | results |
|---|---|---|
| 2 | 1 | >90% polymorph II |
| 1 | 1 | 50−50 polymorph I and II (solvent volume was 1/3 higher than normal) |
| 1 | 2 | mostly polymorph I |
| 0 | 1 | mostly polymorph I |

crystallized in turn acted as seeds, giving the effect of an extreme case of super-seeding.

We put this idea into practice and developed a very reliable crystallization process on a laboratory scale to generate desired meta-stable polymorph I, using less than 5% seeds (as little as 0.5% was also demonstrated in the lab). This process not only assured generation of Form I (even in the area contaminated with Form II) during recrystallization, but it was also used to generate Form I, starting with 100% Form II. We also discovered that by choosing an appropriate ratio of solvent to antisolvent the equilibrium leading to conversion of Form I to Form II could also be controlled with time.

The typical process is described as follows:

Charge 1 kg of ritonavir to reactor A. Then charge 4 L of ethyl acetate to the reactor and reflux until all the solids dissolve. Charge 0.005 kg of seed crystals (of Form I) to reactor B. Charge 4 L of heptanes to reactor B and agitate at ambient temperature.

Slowly filter, using 0.2 $\mu$m filter cartridge, the hot solution (ritonavir in ethyl acetate) from reactor A to reactor B (containing seed crystals of Form I as a slurry in heptanes) over NLT 2 h. (It is not critical to maintain any particular temperature).

Note: An initial slower addition will increase the chance of success. Cool the slurry in reactor B to an ambient temperature, agitate for NLT 3 h, filter, wash with heptanes, and dry. Following this process we were consistently successful in obtaining Form I.

**Solvent Ratio Effects on Rates of Equilibration.** To reduce the propagation of polymorph I to polymorph II, an equilibration study was carried out with different solvent ratios (ethyl acetate:heptanes) at room temperature.

The results are as follows (Table 3):

Isolated polymorph I was contaminated with 1% polymorph II and stirred at room temperature for 21 h.

Conclusion: Equilibration from polymorph I to polymorph II is reduced as the percentage of heptanes increases.

**Manufacturing Process to Control Form I.** From lab studies it was clear that Form I could be generated during crystallization as long as the solution and the reactor were free of any Form II contamination. Form I, being the kinetic form, will always crystallize out first, and seeding with Form I crystals free of Form II could further control this. The following process was successfully developed and implemented in the manufacturing of the bulk drug to consistently obtain the crystalline product with less than 3% Form II contamination:

A solution of ritonavir in ethyl acetate was refluxed for at least 1 h. To this heptanes were charged at the rate to control reflux. After the addition of heptanes was over, reflux was continued for at least 2 h. The solution was cooled to 45 °C and seeded with crystalline ritonavir (Form I) and then stirred for not less than 3 h at that temperature. This was then cooled to 22 °C at the rate of not more than 8 °C/h. The solids were filtered at 22 °C.

**Manufacturing Process to Control Form II.** We also have designed a manufacturing process to produce exclusively the thermodynamically stable Form II, which is described below.

A solution of ritonavir in ethyl acetate was heated to 70 °C. It was filtered and cooled to 52 °C (rate 2−10 °C/h) and seeded with Form II crystals of ritonavir and agitated for not less than 1 h at 52 °C. It was then cooled to 40 °C (rate 10 °C/h) and heptanes was charged and the mixture cooled to 25 °C and stirred for 12 h and filtered. It was dried at 55 °C for 18 h to give exclusively Form II.

## Summary

Although the polymorphism phenomenon is not new to the pharmaceutical and chemical field, Mother Nature continues to surprise the scientific community. One cannot be too careful in dealing with crystalline pharmaceutical bulk drug substances.[6] It is highly advisable to put enough resources to carry on exhaustive research to identify the most stable and all possible polymorphs. Moral of the story is: Dealing with **P**olymorphs is **P**otentially **P**recarious **P**ractice and the **P**roper way to **P**lay this game is with **P**atience and **P**erseverance.

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Department of Chemistry and Chemical Biology

Harvard University

**Abstract.** Microorganisms like bacteria, archaea, and fungi have amazing chemical capabilities, performing challenging transformations and producing complex, biologically active molecules not easily accessed via any other means. However, we do not yet have an understanding of the enzymatic chemistry responsible for the full diversity of reactions performed by these organisms and we lack a complete understanding of the roles this chemistry plays in natural microbial habitats. Recent advances in DNA sequencing technologies have delivered a wealth of microbial genomes, providing an unprecedented opportunity to discover both enzymes that have the potential to change the way chemists make molecules, as well as metabolic activities that will transform our understanding of microbial and human biology. This talk will discuss my group's recent progress in deciphering the enzymatic chemistry responsible for the production of multiple bacterial metabolites, including scaffolds of unusual molecular architecture and disease-linked molecules made by the human gut microbiota.

**Thursday, March 3, 2016**

**Cram Conference Room – 3440 Molecular Sciences Bldg**

**Attachments** 2016-03-03 Balskus and Caille Amgen Lectureship Series flyer.pdf

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# Amgen Drug Substance Technologies: A Process to Manufacture AMG 232

March 2016

# Modality Independence at Amgen

Amgen is a modality-independent biopharmaceutical company whose product portfolio and clinical pipeline showcase the use of eight different modalities.

These modalities include therapeutic proteins, monoclonal antibodies, fusion proteins, BITE® antibody-constructs, antibody drug-conjugates, and small molecules.

Small molecules, manufactured entirely by synthetic means, account for a continuously increasing portion of Amgen's revenues.

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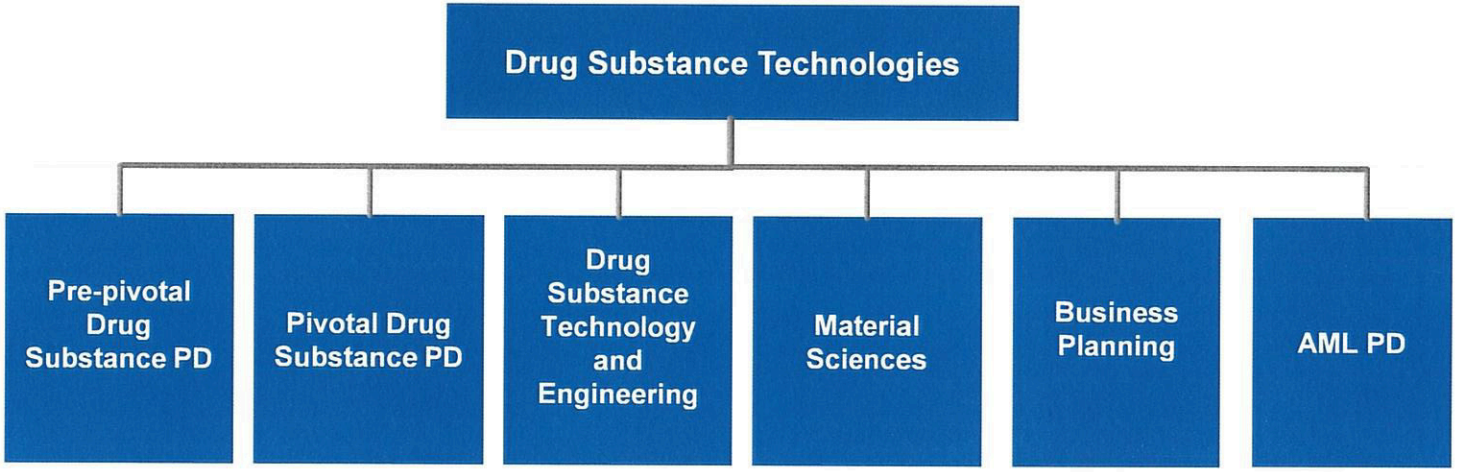
# Drug Substance Technologies

*Integrated team consisting of both synthetic and biological groups that enables fast to clinic approaches as well as developing state-of-the-art processes*




***Pre-Pivotal Drug Substance Process Development***

*Activities include development of processes for all molecules entering pre-pivotal clinical testing, as well as molecular biology, cell line development and genetic stability characterization of cell banks*

***Pivotal Drug Substance Process Development***

*Activities include commercial process development, process characterization, and support of tech transfer to the commercial network*

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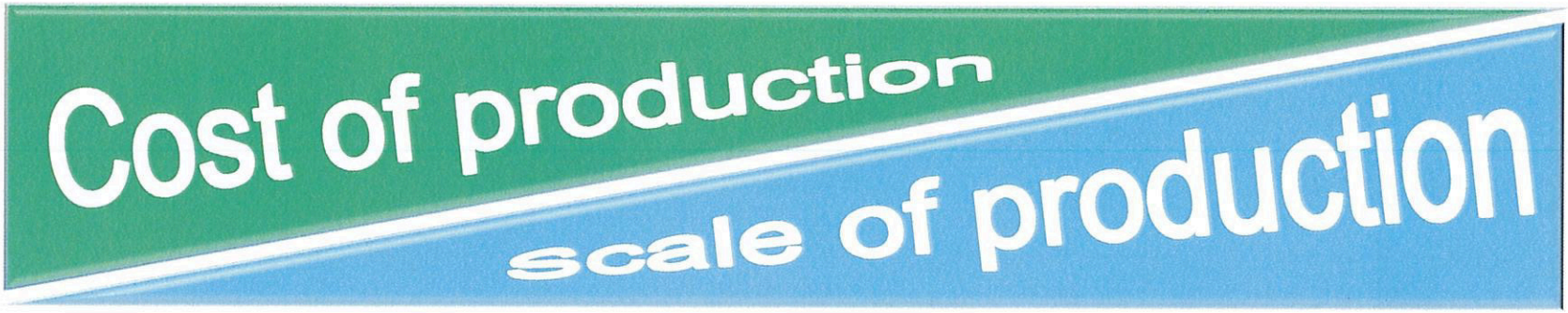
# Drivers Change During Development

<$100,000 / kg <$2000 / kg




grams tons

- **Early focus is on speed to clinic as main driver of development**
  - Develop robust process, make it safe, ensure quality
- **For commercial development, efficiency and continuous supply are main drivers**
  - Control the process, reduce waste, ensure quality

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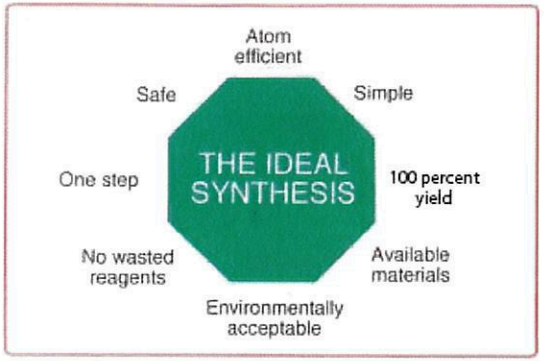
# Synthetic Design Criteria

Often a decision between various potential manufacturing routes is required. A number of criteria are taken into account to choose the commercial route including:

1. Material Cost
2. Yield
3. Volume-Time-Output
4. Environmental Factor / Process Mass Intensity
5. Quality
6. Reproducibility / Robustness

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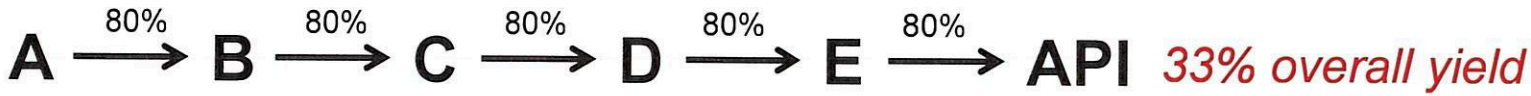
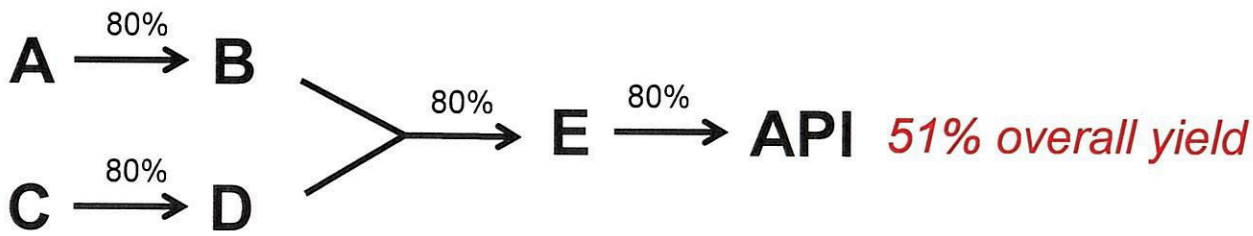
# Designing the Synthetic Route

- **What can be bought?**
- **How can it be put together?**

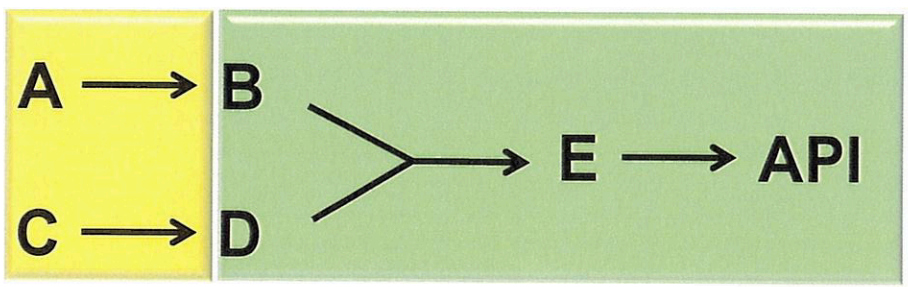
*Linear Synthetic Route*




*Convergent Synthetic Route*




- **How will quality be controlled?**




***Manufacture under cGMPs***

- Quality is designed into the process (QbD)
- In-process tests used to monitor process
- Release testing of final product (CoA)

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# Can the route be safely scaled?

- **How much energy does the reaction generate?**
  - Do you have adequate cooling or heating capacity
- **How is the reaction controlled?**
  - Can reaction rate be controlled by reagent addition rate?
  - Do reactive intermediates accumulate?
- **What are the by-products?**
  - Are gasses generated?
    - What are they?
    - How to scrub?
  - Are secondary reactions possible?
- **How toxic are the reagents / products / by-products?**
  - Can they be avoided?
  - How do you prevent exposure, ensure containment?
- **What happens if something goes wrong?**
  - Add too much or too little reagent
  - Over heat or under heat
  - Poor mixing
  - Cooling / heating failure
  - Unanticipated operator error?

**Understanding the reaction mechanism and failure modes ensures safety**

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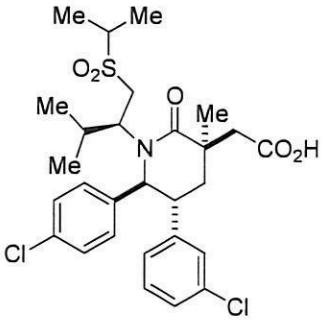
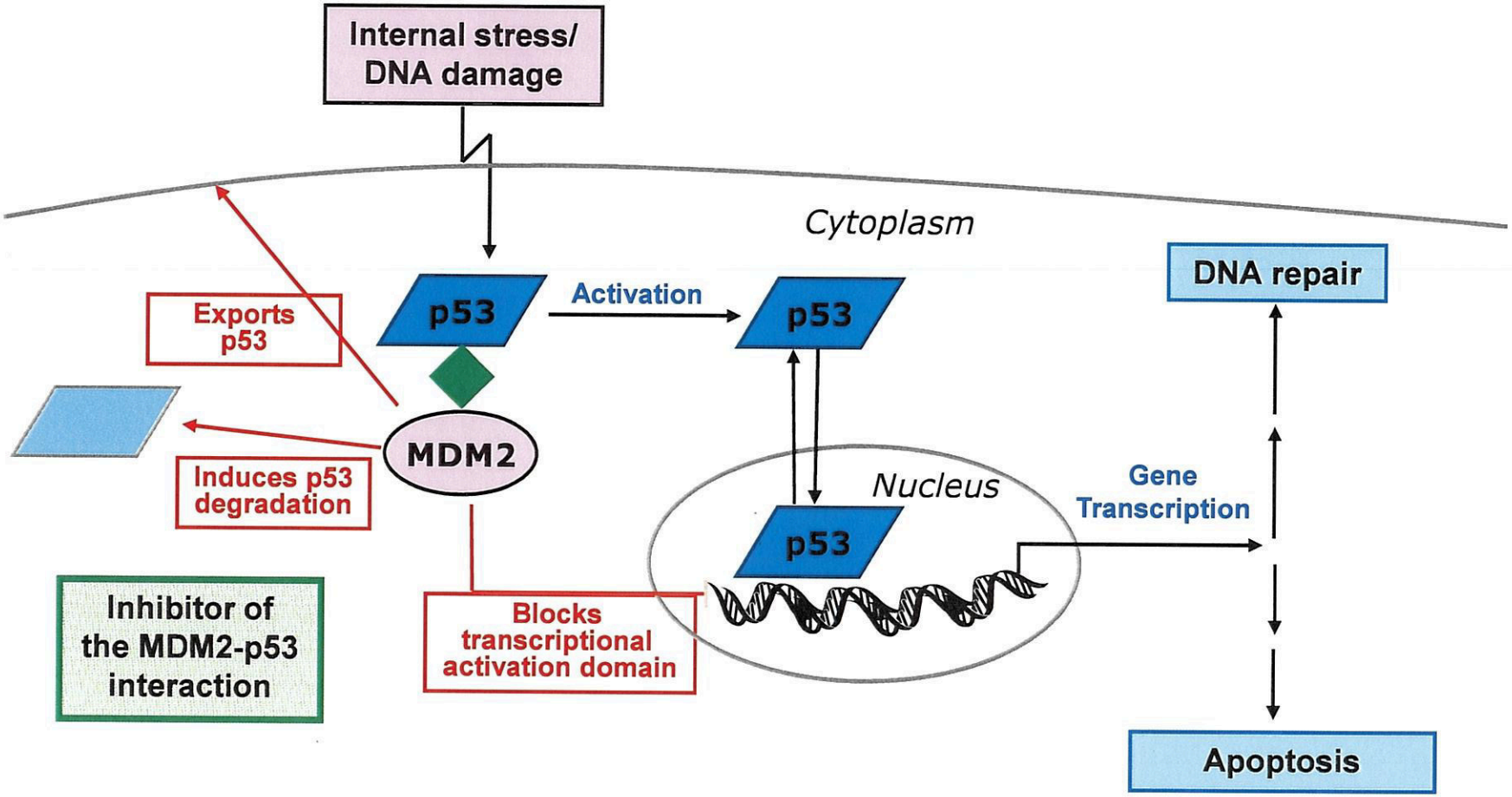
# AMG 232
# Process Development




AMG 232

# MDM2 Represses the Activity of p53 Tumor Suppressor




Chene, P. *Nature Reviews Cancer,* **2003**, *3*, 102-109.



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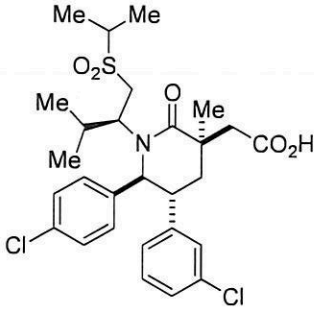
# AMG 232 Program Overview

- Oncology Indication: Inhibition of the MDM2-p53 protein-protein interaction
- MDM2 represses the activity of p53 tumor suppressor
- FIH in Q4 2012
- Multiple clinical trials ongoing and planned in 2015-2018
- Drug substance form: crystalline anhydrous free acid




AMG 232

Daqing Sun-Steve Olson, coworkers, *J. Med. Chem.*, **2014**, 1454.

## Presentation Overview

i. Manufacturing Strategy

ii. Process Development:

a) Sulfone formation

b) Ozonolysis/Pinnick Oxidation

c) Final Form setting/Control of Drug Substance Quality Attributes



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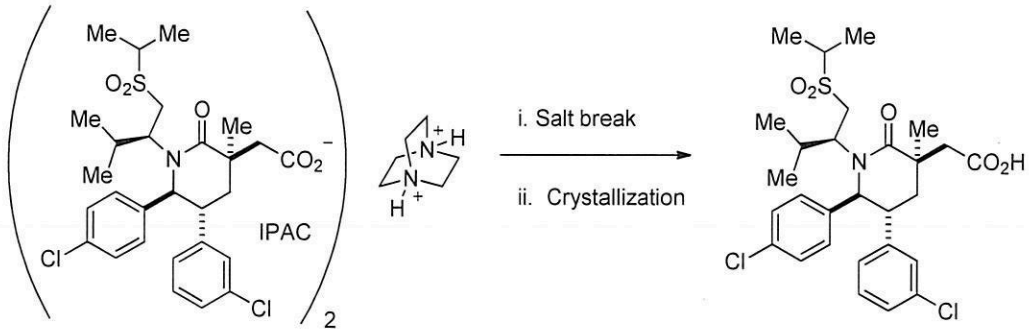
# Manufacturing Strategy

- Clinical manufacturing process has been developed internally and demonstrated at CMO




AMG 232 DABCO Salt
**Purity Gate Keeper**

AMG 232 Anhydrous

Crude Isolation of AMG 232 as a salt acts as ***purity gatekeeper*** and decouples the process from DS QAs

## *Process Intensification towards Commercial Process*

- Using same process intermediates, optimize other process inputs (reagents/solvents/CM vs batch)
- Objective is to minimize COG by
  - ⇒ Minimizing raw material costs/overall cycle-time/# of unit operations while meeting DS CQAs
  - ⇒ Perform PC and engineering runs



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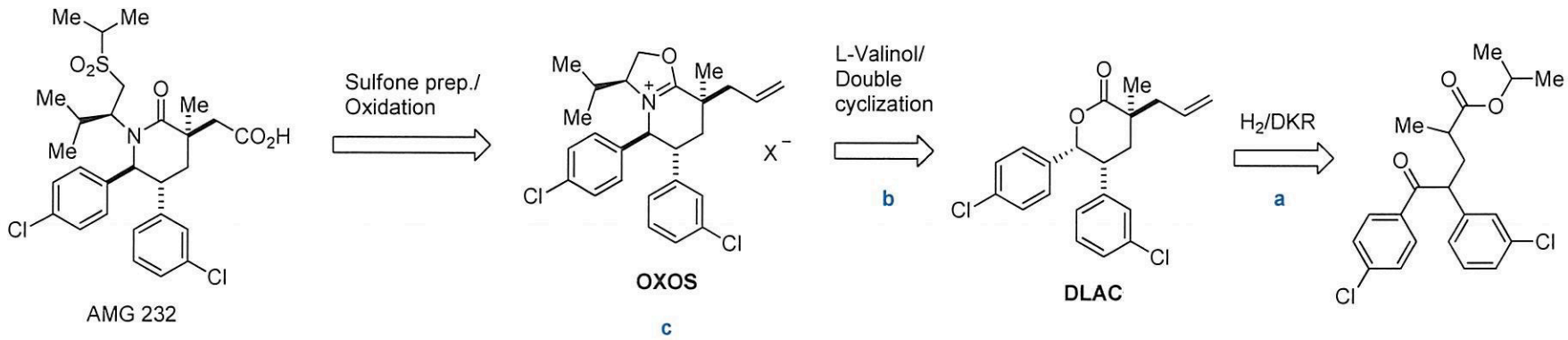
# AMG 232 Retrosynthetic Analysis




- Access to OXOS has been devised by Amgen Discovery group and covered in:

  Brian Lucas-Steve Olson, coworkers, *JACS*, **2012**, 12855.

- Subsequent Route improvements include:
  a. A robust DKR process/Identification of a new catalyst
  c. A non-cryogenic access to **OXOS** by double cyclization (with $Ts_2O$)
  d. A naphthyl sulfonate ($X^-$) salt (**OXOS**) as an efficient crystalline purity gatekeeper



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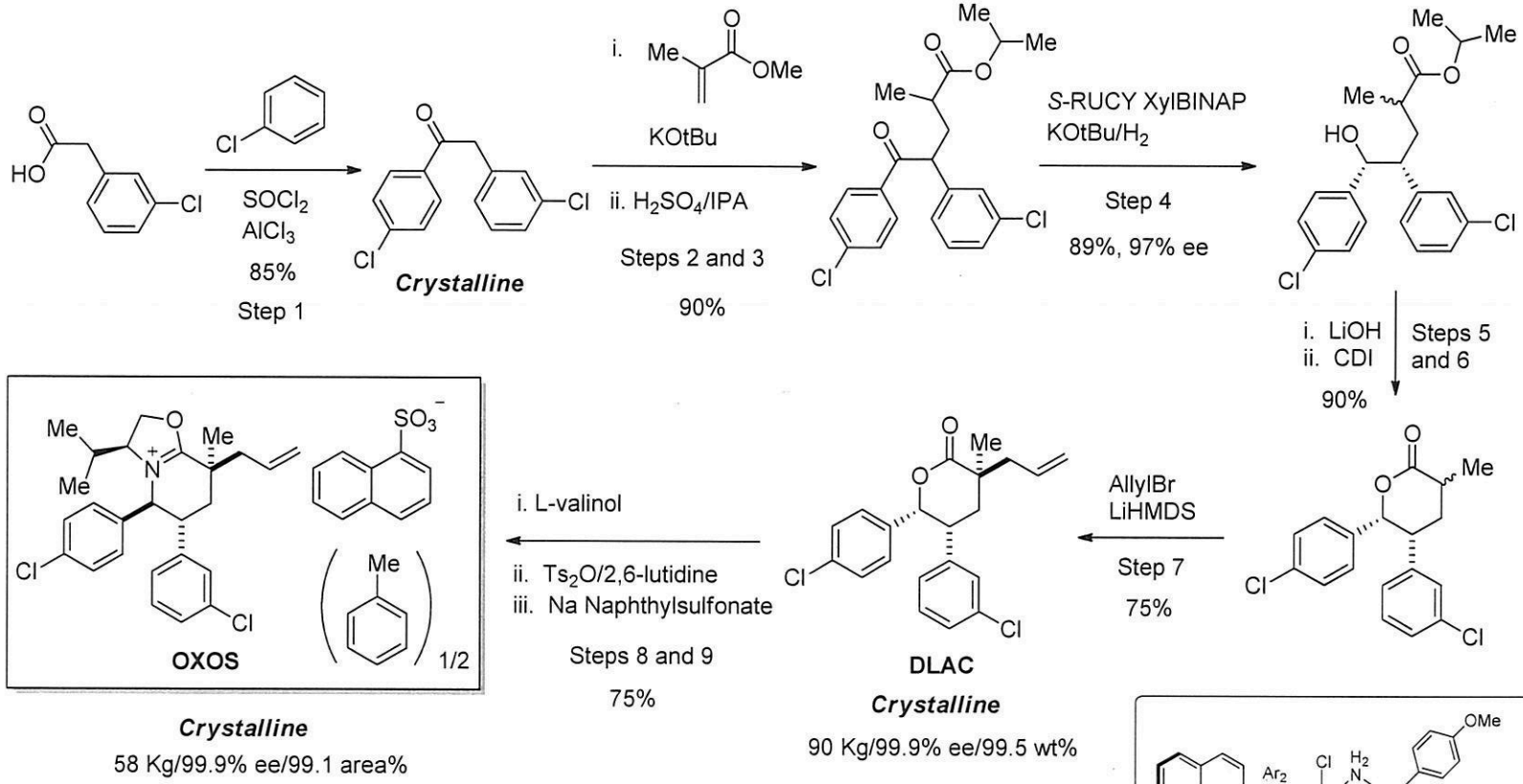
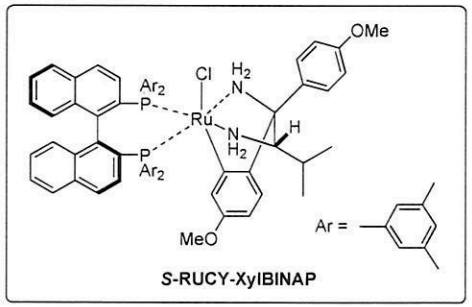
# OXOS Manufacturing Process




- **OXOS**: 32% yield from raw materials
- RUCY catalyst (0.05 mol%) affords 97% ee



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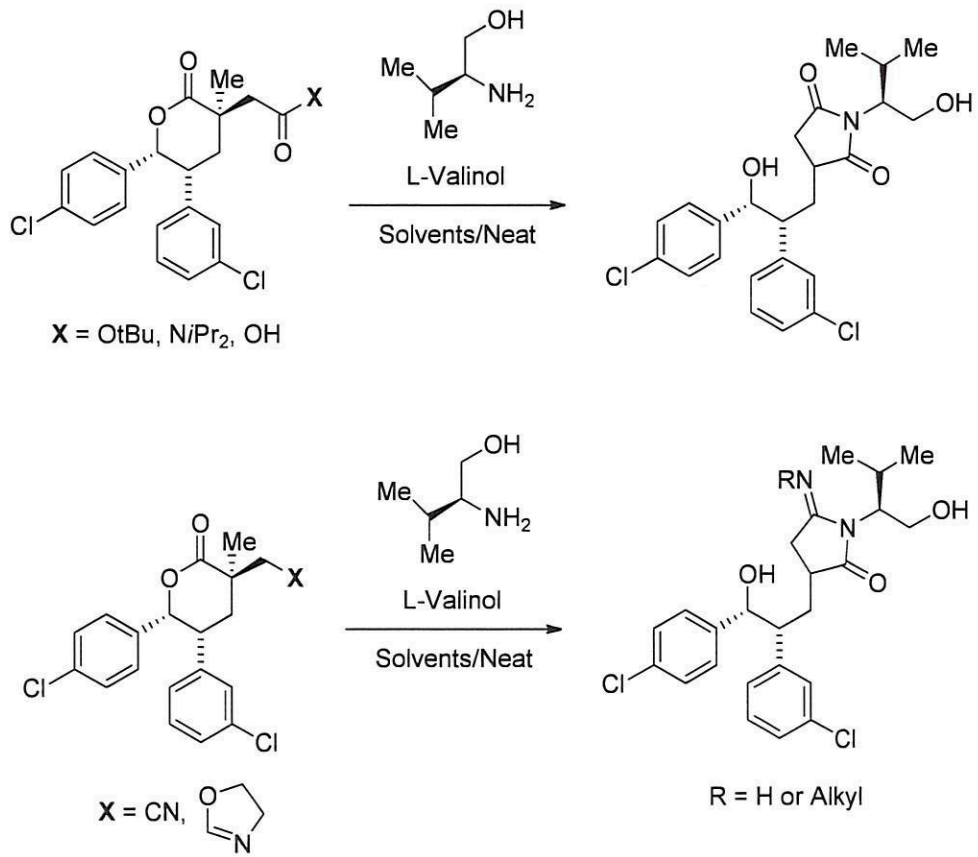
# Side-Chain Route Selection




- Performing lactone ring-opening with following side-chain group:

  i. Ester
  ii. Acid
  iii. Amide
  iv. Nitrile
  v. Oxazoline

  leads to formation of succinimide derivatives as major product

***Elimination of Oxidation of Allyl to Acid would Simplify the Process***



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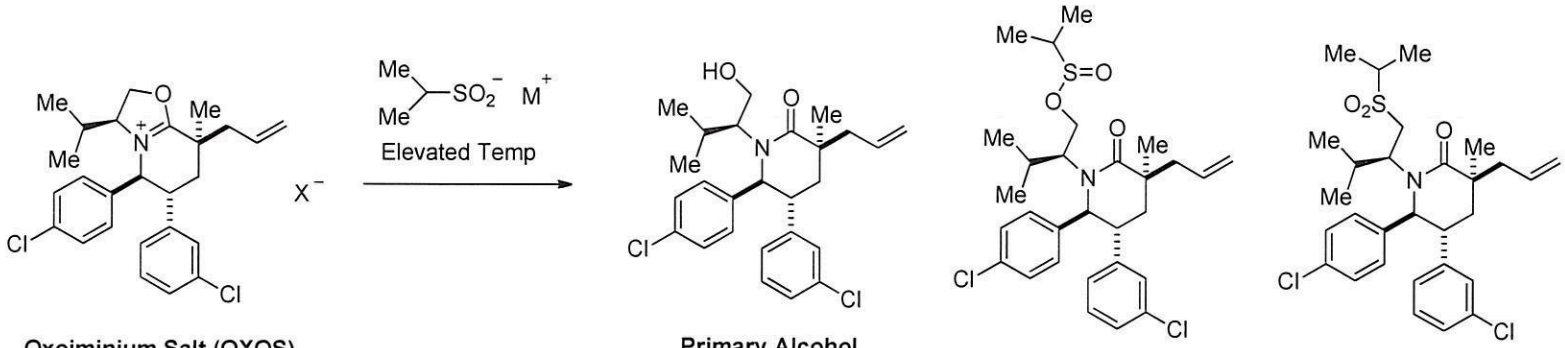
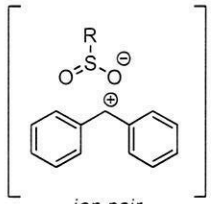
# Allyl Sulfone Manufacture from Oxoiminium Salt

- Direct Manufacture of sulfone (as opposed to thioether) is preferred option for AMG 232 prep.
- Avoids thiol stench and sulfoxide to sulfone oxidation




- Rearrangement of benzhydryl sulfinate esters to sulfones have been described to take place by **ion pair formation/recombination mechanism**:
  Braverman *et al.*, *Tetrahedron*, **1990**, 2979 and Mayr *et al.*, *JACS*, **2010**, 4796.




- For 232 system, sulfinate ester to sulfone rearrangement is likely to involve **dissociated ions**:
  ⇒ Stability of **OXOS**/concentration dependence/cross-over experiment with $EtSO_2MgCl$



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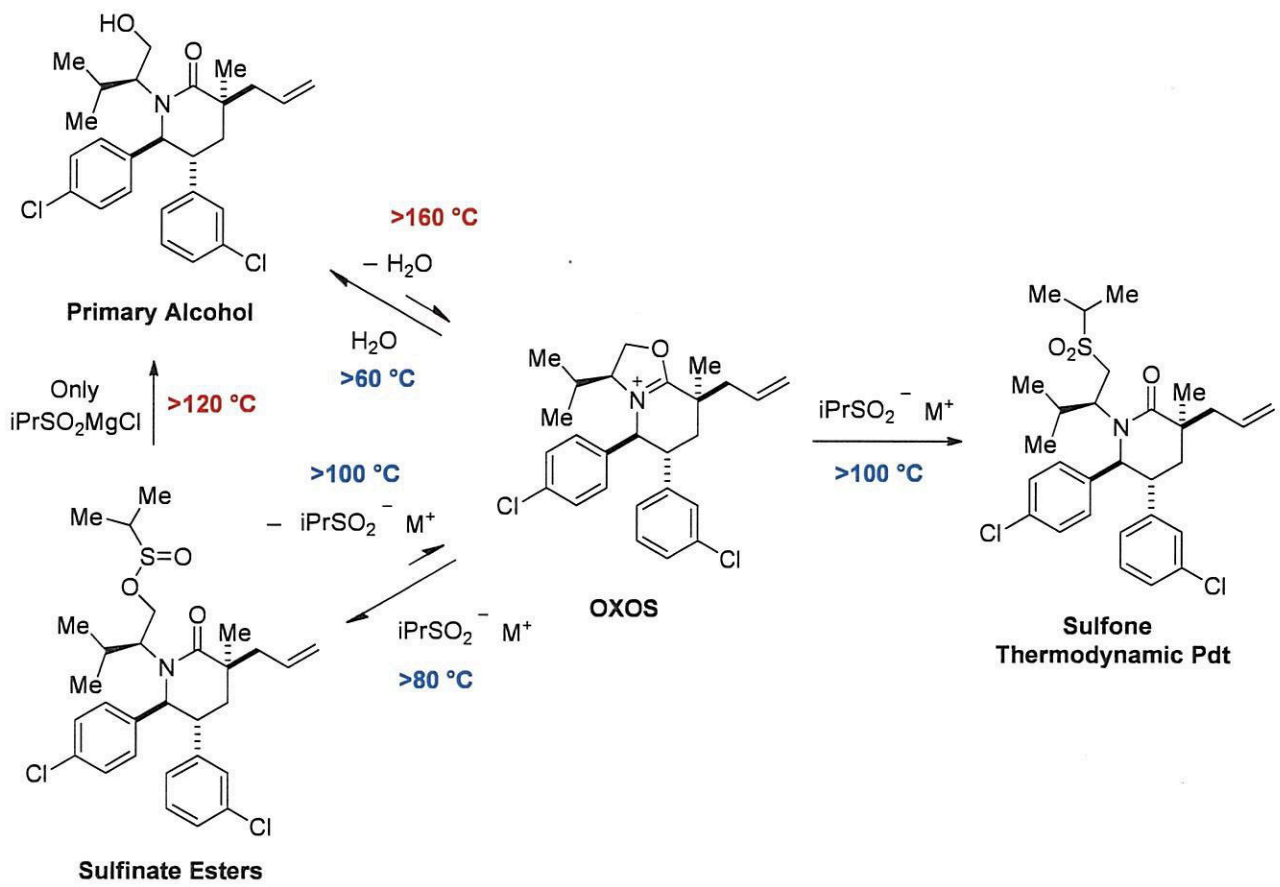
# Proposed Mechanistic Relationships




**Two available options to manufacture Sulfone:**
**i. Operate under rigorously water free conditions**
**ii. Operate at >160 °C: Primary alcohol becomes a competent intermediate**



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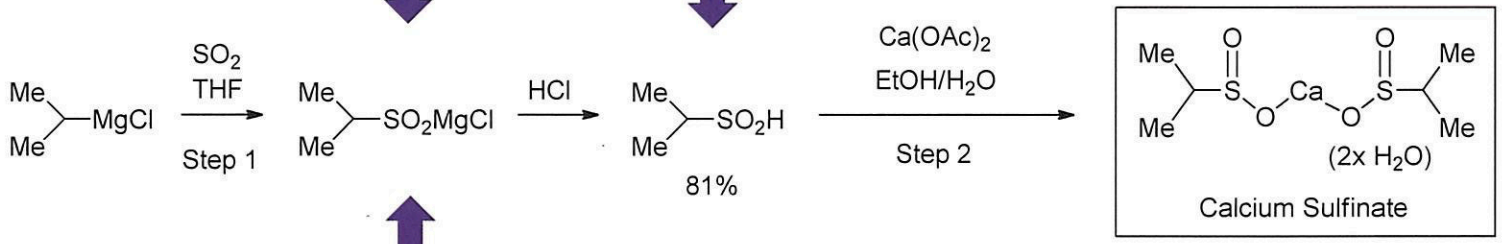
# Selection of Isopropyl Sulfinate Reagent

i. Oil: challenging to release in GMP process
ii. Poor stability to azeotropic drying process (disproportionation)

i. *In-situ* preparation

i. *In-situ* preparation of Na salt in AS step




Isolated Crystalline Dihydrate
75%

i. Control of Stoichiometry of $SO_2$ and iPrMgCl to avoid impurity formation
ii. Non-water related Alcohol access

i. Prepared in 2 steps
ii. Isolated as Hydrate
iii. Poor solubility for CM

i. Stable Crystalline solid can be shipped to manufacturing site and release in GMP process
ii. Stable to in-situ azeotropic drying



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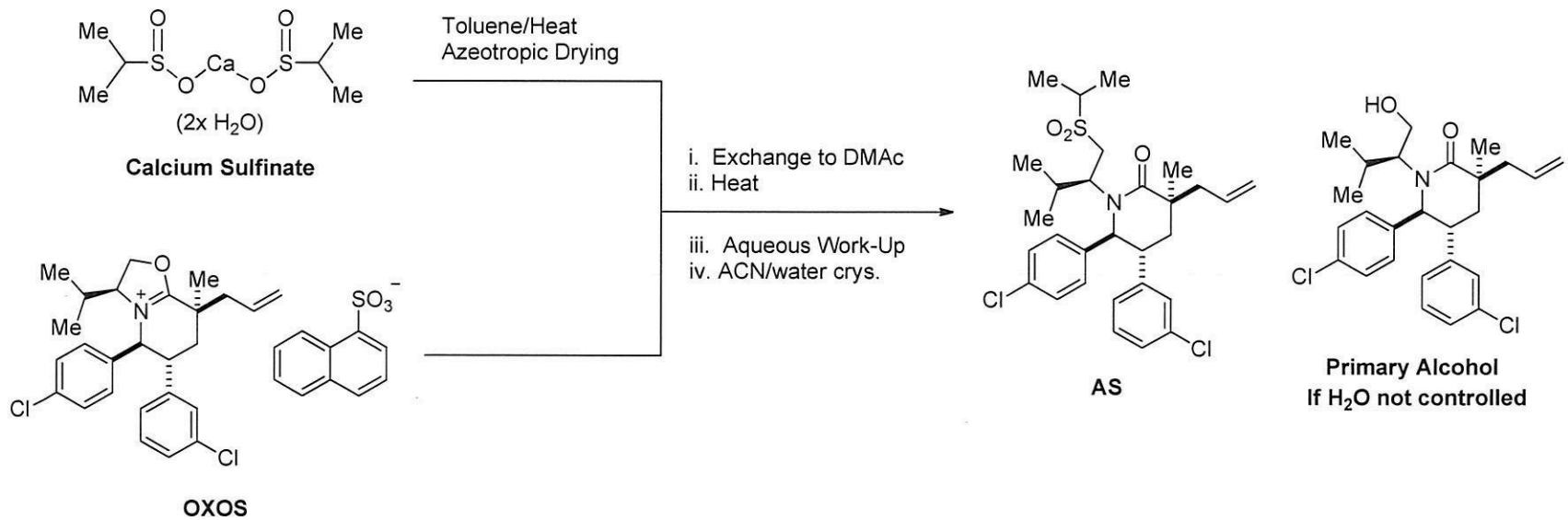
# Manufacture of AS from OXOS and Ca Sulfinate




## Development of a Process at ≤120 °C using Rigorous Drying of Reaction Mixture

i. Calcium Sulfinate Dried by Azeotropic Distillation/Toluene
ii. OXOS added as a solid
**iii. Ca Sulfinate not soluble in toluene: Productive chemistry start upon solvent exchange to DMAc**
iv. Solvent exchange to DMAc

4 batches manufactured at Amgen:
2-5 kg batches/99.2-99.6 LCAP/82-83% PAY

***Filisaty Vounatsos***



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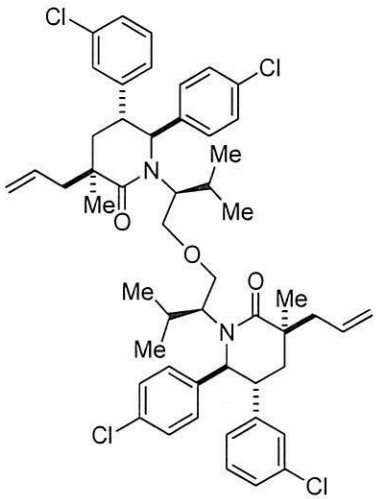
# Stability of mixture of OXOS/Ca Sulfinate at Elevated Temperature

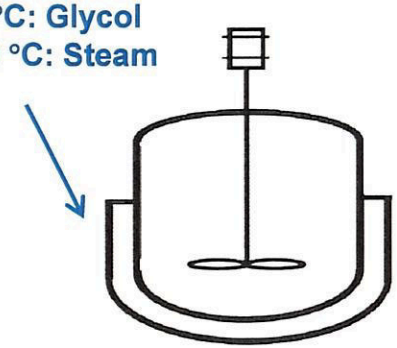



***Ether Impurity Isolated and Identified by $^1$H NMR and MS***

| Time (h) | Temp (°C) | % Tol (by volume) | % DMAc (by volume) | RRT 1.3 LCAP |
|---|---|---|---|---|
| 6 | 110 | 100 | 0 | 8.0 |
| 6 | 100 | 100 | 0 | 3.5 |
| 6 | 80 | 100 | 0 | ND |
| 6 | 110 | 65 | 35 | 0.3 |
| 6 | 80 | 65 | 35 | ND |

i. Ca Sulfinate decomposition pdts slowly solubilize in toluene phase and react with OXOS giving rise to ether impurity (RRT 1.3)
ii. Ether impurity is a thermal hazard that does not convert to product at 120 °C
iii. Does not form at ≤80 °C or at ≤65% toluene v/v

**≤90 °C: Glycol**
**≥100 °C: Steam**



**Jacket temperature control switches from glycol to steam between 90-100 °C so a rapid solvent ex. in this temp range cannot be operated**
**⇒ DMAc must be added to the mixture before jacket switch**



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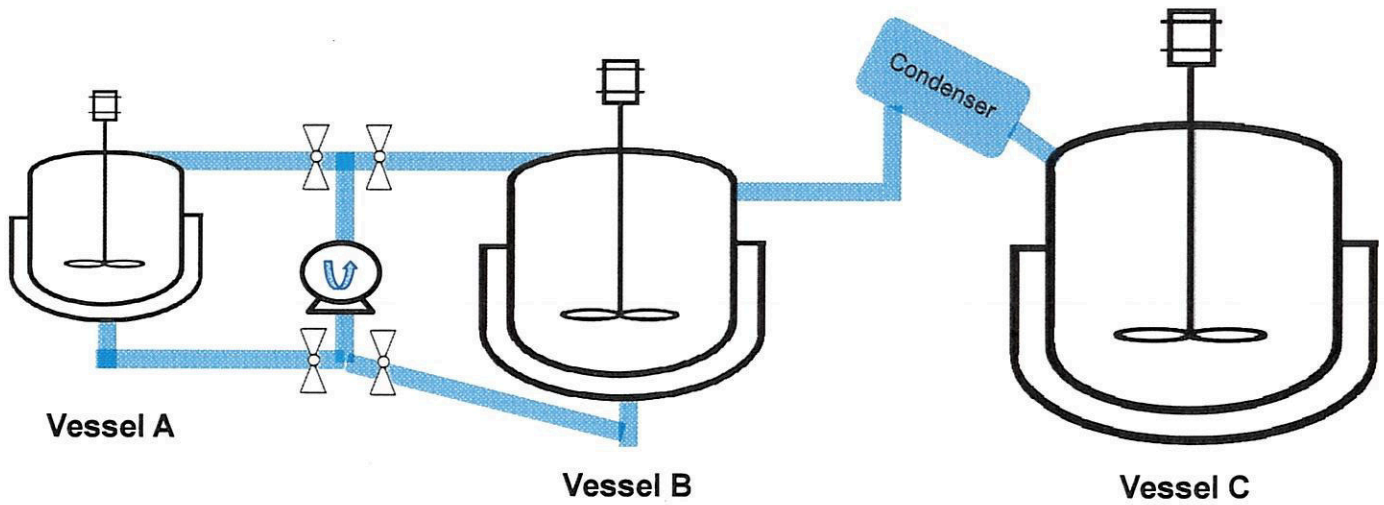
# Manufacture of AS from OXOS at CMO




Cal. Sul. Azeotropic Drying in vessel B (90-100 °C)

Transfer of slurry to vessel A

→

OXOS Azeotropic Drying in vessel B (<80 °C)

Transfer of Cal. Sul. slurry to vessel B

→

Combined slurry concentration in vessel B (<80 °C)

~1/2 DMAC added: mixture is >35% v/v DMAc

→

i. Jacket switch from glycol to steam

ii. Collect distillate

→

i. ~1/2 DMAC added

ii. Collect distillate (110-120 °C)

**CMO Results: ~20 Kg/99.3 LCAP/83% PAY; no RRT 1.3 impurity generated**



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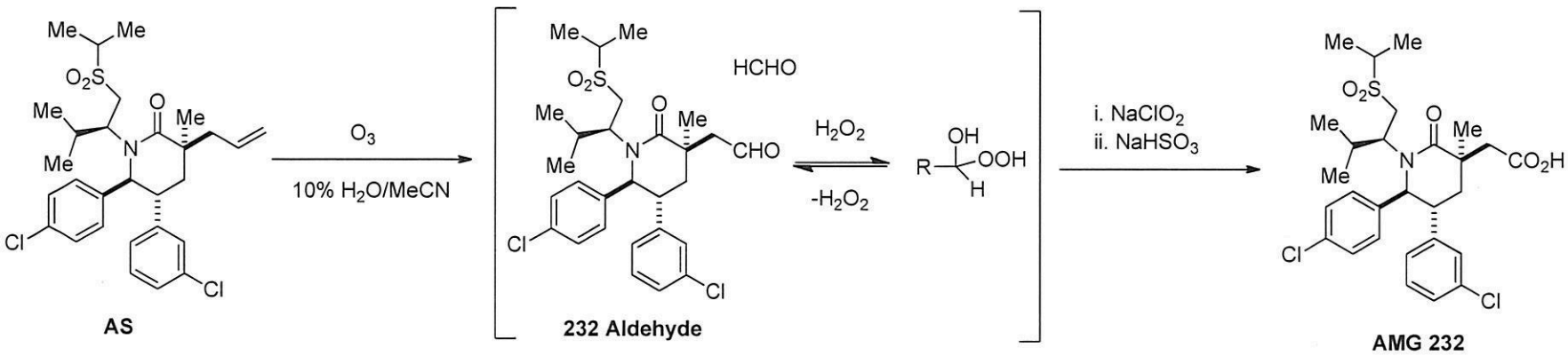
# AMG 232 via Ozonolysis-Pinnick Oxidation



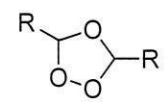

- Early clinical deliveries utilized $RuCl_3/NaIO_4$: Impurity profile and downstream treatment challenges
- Ozone/$NaClO_2$ robust process that offered >95 area% AMG 232 and simple downstream handling
- Ozonolysis ran in $H_2O$/Acetonitrile mixture (Dussault *et al*, *JOC*, **2008**, 4688) :

  ⇒ No accumulation of ozonide: aldehyde and peroxy-acetal in solution

  ⇒ Reaction mixture studied by ARC: 92 J/g <240 °C-no safety concern

  ⇒ Experimental LOC is 10.75 vol%

  ⇒ Decision to operate at ½ LOC: ~5 vol% oxygen



**Ozonide**

| $H_2O$ | LCAP |
|---|---|
| 10 % | 1.3 % |
| 5 % | 2.8 % |
| 2.5 % | 3.2 % |
| 1 % | 11.7 % |



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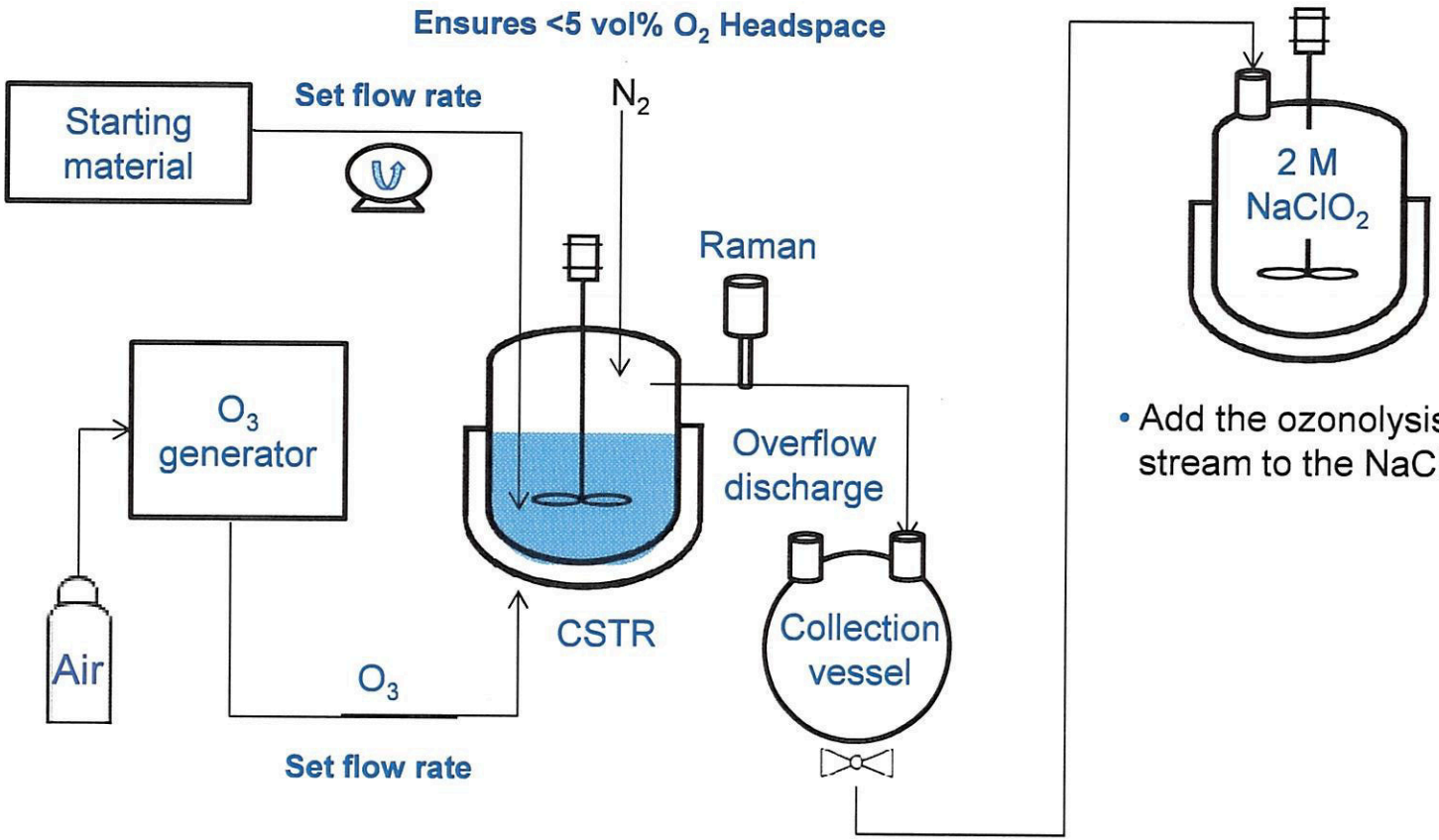
# CSTR Ozonolysis/Pinnick Apparatus




- Add the ozonolysis reaction stream to the $NaClO_2$ quench

- Excess ozone utilized (2.5 equivalents)/AS <0.2 area%/Original AMG 232 isolation did not tolerate AS
- 95% SAY/4.8 kg AS processed GMP in 30 h (GMP)
- Allian *et al.*, *OPRD*, **2011**, 91-97.

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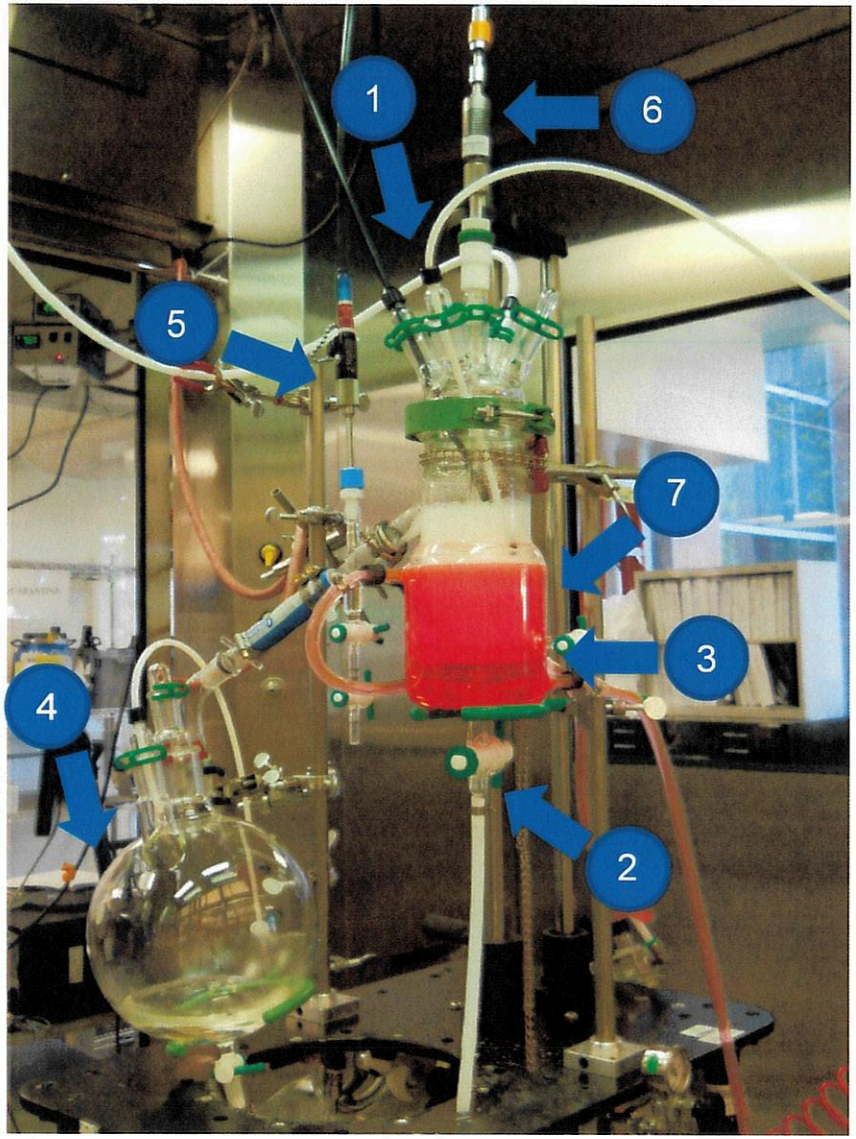
# CSTR and Ozone Generator




1. Starting material inlet
2. Ozone inlet
3. Glass frit for finely dispersed bubbles
4. Receiving flask
5. Raman probe
6. Propeller shaft
7. Jacket

***Ozone Generator***

(~1 mol $O_3$/h)

***Brian Cochran***

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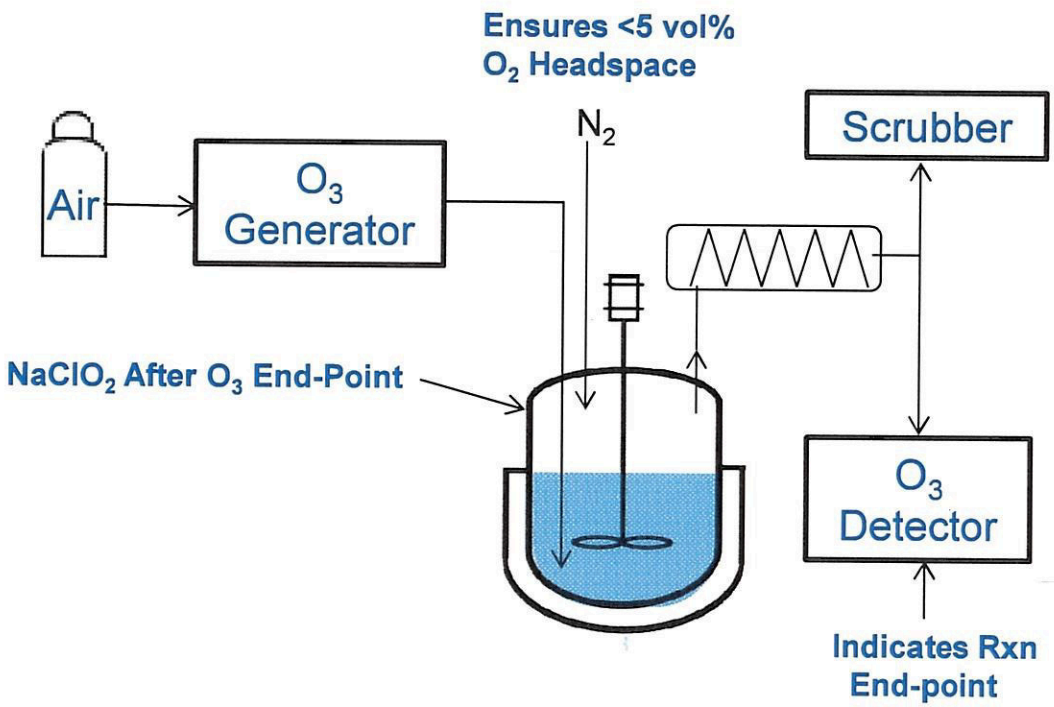
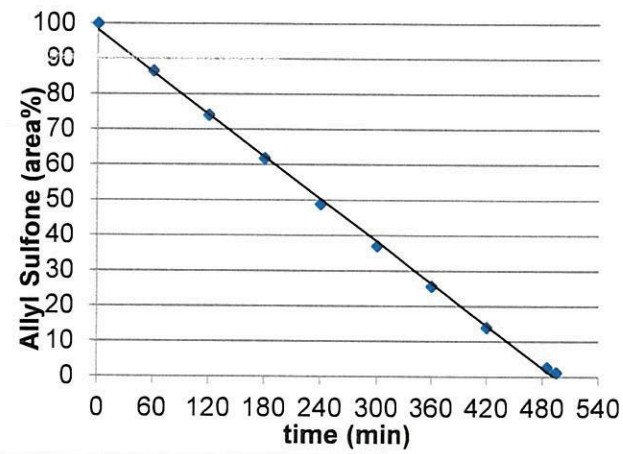
# Semi-Batch Ozonolysis/Pinnick Apparatus




Linear AS consumption (HPLC)

- Excess ozone utilized (1.15 equivalents)/~1 area% AS
- 96% SAY/8.0 kg AS processed GMP in 20 h (GMP)



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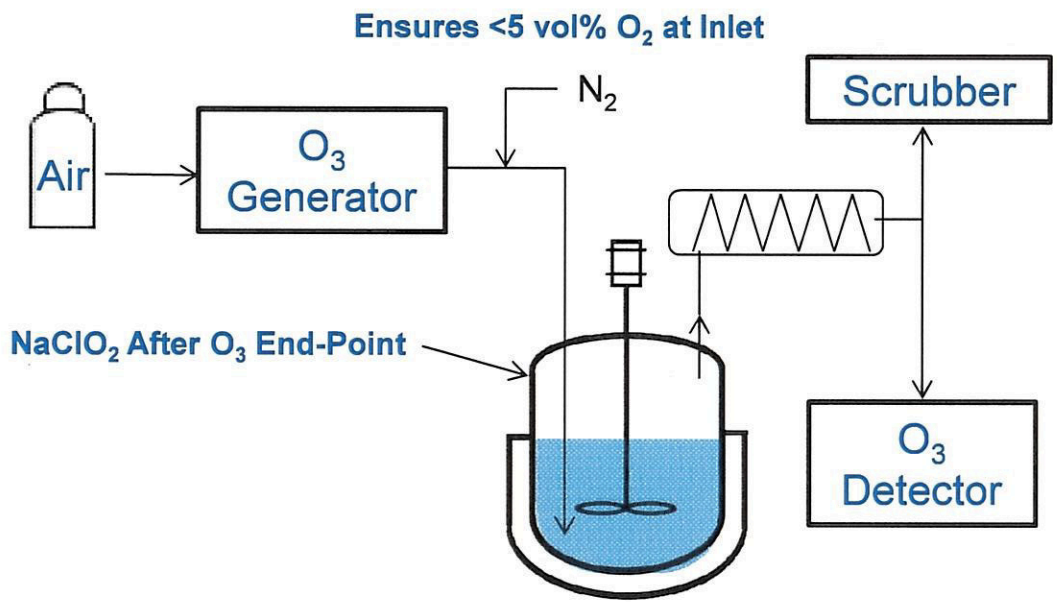
# Use of Dilute Air at CMO

- CMO used mixing of Air from the $O_3$ generator with $N_2$ at inlet to ensure <5 vol% $O_2$ in reaction mixture and mitigate explosive hazard
- CMO selected primarily for hazardous material handling capacity/no vessel designed for ozonolysis
- Portable gas inlet (sparger) is used




***The amount of gas passing through the sparger is 4x greater, crystallization of SM/products occurs via: i pressure drop; ii. sparger cooling; iii. crystallization in sparger; iv. pressure drop***

# Pierced Hastelloy Tube Solution

**Original Sparger**

**Pierced Sparger**

- *1/8*-inch diameter holes pierced in C22 Hastelloy tube (37 holes)
- No pressure drop at desired gas flow/no crys of material in sparger
- Sparger surface temp close to reaction mixture
- Process ran at 30 °C instead of 20 °C to increase solubility of SM/pdts
- Despite larger bubble size/1.11 equivalents $O_3$ to reach completion
- ~20 kg AS processed in 8 h/~1 area% AS/97% SAY

**Original Sparger (measured in 30 L chemglass vessel)**

| T Jacket (°C) | Gas flow (scfm) | T reaction (°C) | T sparger (°C) |
|---|---|---|---|
| 35 °C | 11 scfm | 12 °C | 2 °C |
| 80 °C | 11 scfm | 30 °C | 11 °C |

**Pierced Hastelloy (measured in 30 L chemglass vessel)**

| T Jacket (°C) | Gas flow (scfm) | T reaction (°C) | T sparger (°C) |
|---|---|---|---|
| 35 °C | 11 scfm | 13 °C | 11 °C |
| 70 °C | 11 scfm | 25 °C | 22 °C |



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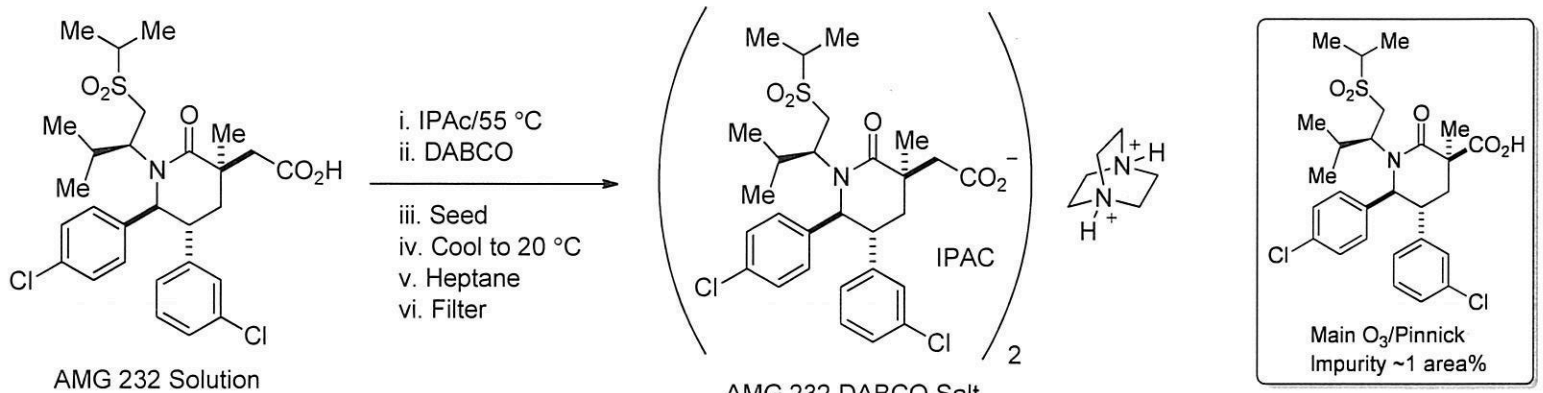
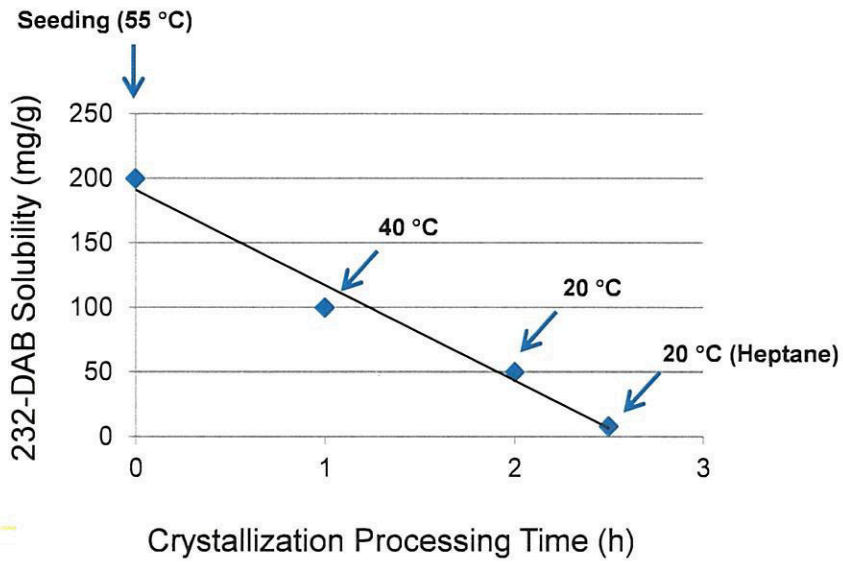
# AMG 232 Crude Isolation as DABCO Salt




AMG 232 Solution
96 area%

AMG 232 DABCO Salt
(**AMG 232-DAB**)

**CMO Manufacture:**
**~20 kg/99.95 area%/**
**78.5 wt%/82% PAY**




- Anhydrous AMG 232 poorly rejects process impurities
- >35 salts prepared and DABCO salt selected as purity gatekeeper (controls DS QAs)
- Suitable for crystallization design
- Excellent impurity rejection



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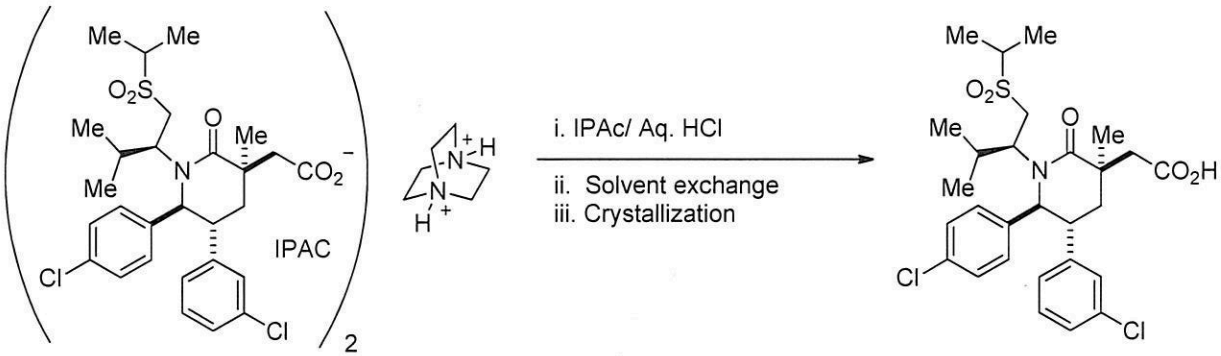
# Final Form Crystallization




AMG 232 DABCO Salt
(**AMG 232-DAB**)

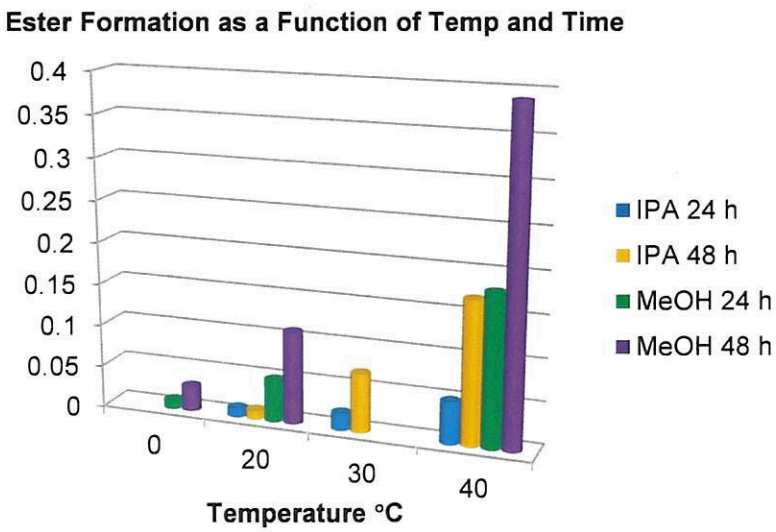
AMG 232 Anhydrous

- Orthogonal aqueous system sought:

  To reject impurities from potential over-oxidation by $O_3$
- Alcohols (MeOH, IPA) are excluded:

  Fisher esterification
- AcOH is a benign solvent to crystallize $CO_2H$ DS

  from if AcOH is cleared efficiently in filtration/washes




*Filisaty Vounatsos*

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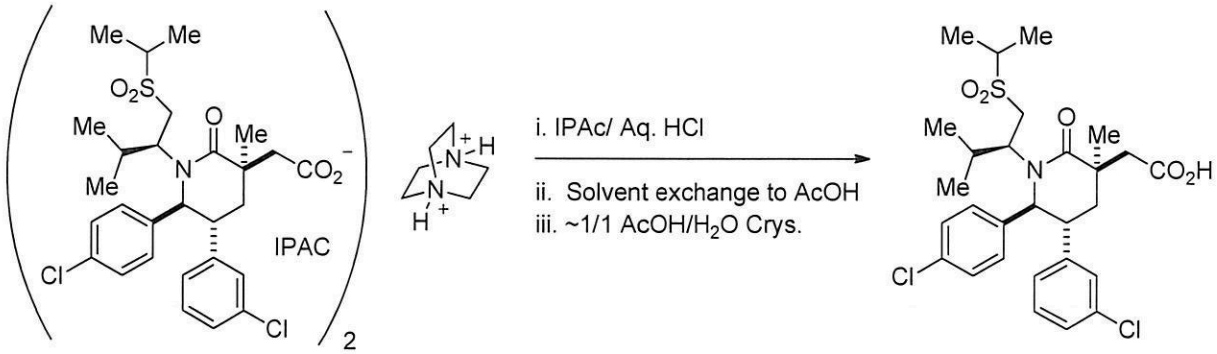
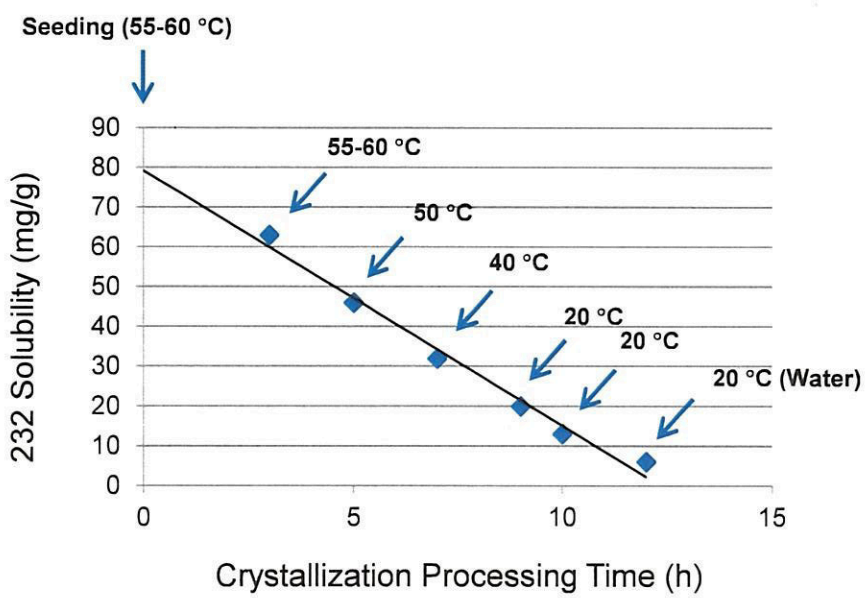
# Final Form Crystallization




AMG 232 DABCO Salt
(**AMG 232-DAB**)
99.95 area%

AMG 232 Anhydrous

**CMO Manufacture:**
**~20 kg/99.95 area%/101 wt%/**
**94% PAY/195 ppm acetic acid**




- Temperature driven crystallization
- Rapid filtration
- 3 water washes reduce AcOH levels
- Milling using Pallman Universal
- Single Crystalline Anhydrous Form
- Short Rods/No agglomeration



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Andreas Reichelt (Research Operations)
Tawnya Flick (AS)
Belle Liu (QA)
Steve Olson
Brian Lucas
ASF Discovery team




AMGEN

# SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

## NIT: 800.176.089-2

## COMPROBANTE DE RECAUDO SISTEMA SIPI

**Radicado: 15293617**
**Recibo: 17-69184**
**Fecha: 31/08/2017**

**CAROLINA MERCEDES DAZA MONTALVO**
**CC: 66.783.790**
**Pago PSE No: 107647**
**CUS: 278920589**

Patente PCT
PROCESOS DE FABRICACIÓN Y FORMAS CRISTALINAS DE UN INHIBIDOR DE MDM2

**Detalle del pago**

| Id. | Rentístico | Concepto y Servicio | Cant. | Val. Unit. | Subtotal |
|---|---|---|---|---|---|
| 1 | 50005-01-01 | Solicitud examen de patentabilidad Patente de Invención (DESCUENTO INTERNET | 1 | $ 996,000 | $ 996,000 |
| | | TOTAL | | | $ 996,000 |