

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

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Doctor

José Luis Salazar

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

E. _____ S. _____ D. _____

Referencia: Expediente No. 07.034.171

Solicitud de Patente de Invención titulada: "Composición que comprende amitraz y un compuesto de hidrazinacarboxamida en una mezcla de disolvente que no tiene grupos hidroxilo"

Solicitante: Wyeth

RESPUESTA AL AUTO NOTIFICADO POR FIJACIÓN EN LISTA NO. 480**DEL 16 DE DICIEMBRE DE 2011, OFICIO NO. 19558 (EXAMEN DE FONDO)**

ÁLVARO CORREA ORDÓÑEZ, mayor de edad y vecino de Bogotá D.C., identificado tal como aparece al pie de mi firma, en mi carácter de apoderado de la sociedad en referencia, doy respuesta al auto emanado por ese Despacho dentro del término legal:

1. Reivindicaciones

Su Despacho menciona que las reivindicaciones 1 a 4 definen claramente los elementos de la composición de interés y sus proporciones de combinación.

Sin embargo, su Despacho solicita que las reivindicaciones 5 y 6 deben retirarse ya que dichas reivindicaciones reclaman una composición tópica que no fue completamente divulgada en la descripción, y por lo tanto no se encuentra enteramente soportada.

Nivel inventivo

Sobre el requisito del nivel inventivo, el Examinador establece que el documento **D1**

(US2004/0116419) afecta el nivel inventivo de las reivindicaciones 1 a 8 de la presente invención.

El Examinador establece en forma resumida:

“.....El documento D1 es el más cercano a la invención definida en la reivindicación 1 porque: D1 divulga una composición que comprende un vehículo farmacéuticamente aceptable y una cantidad eficaz desde el punto de vista ectoparasitocida de un antagonista del canal de sodio neuronal y amitraz. Dicho antagonista corresponde al mismo compuesto parasitocida de esta invención (metaflumizona)....(....)”

En los ejemplos de D1 se divulgan específicamente composiciones parasitocidas que comprenden la mezcla entre amitraz y metaflumizona en altas concentraciones (10% y 20% respectivamente) y que ofrecen un alto porcentaje de eficacia del 99%-100% (tablas III y IV).

Al comparar la materia divulgada en esta solicitud, con la materia conocida desde D1 se observa que la diferencia radica en el sistema solvente, específicamente en la presencia ahora de γ -hexalactona, eucaliptol, y acetato de 1-metoxi-2-propilo. Sin embargo, dicha diferencia no provoca ningún efecto técnico, puesto que los porcentajes de eficacia y estabilidad en los mismos intervalos de tiempo en ambos casos son igualmente altos... (...).

Se le aclara al solicitante que si bien es cierto, los datos de la tabla I de esta solicitud muestran una alta eficacia (98.8) hasta 28 días y (95.9) hasta 35 días después del tratamiento, los datos de las tablas III y IV del documento D1 también muestran una alta eficacia (100%) hasta los 28 días después del tratamiento. Y teniendo en cuenta además que D1 divulga específicamente composiciones veterinarias para aplicación tópica para el control de plagas que incluyen la combinación entre amitraz y metaflumizona en altas concentraciones y en un sistema solvente libre de grupos hidroxilo, se concluye que la composición reclamada en esta solicitud se deriva de manera evidente de D1 y definitivamente no tiene nivel inventivo”.

2. Argumentos a favor de la patentabilidad

2.1 Claridad de las reivindicaciones

Se presenta un nuevo pliego reivindicatorio, en el cual se cancelaron las reivindicaciones 5 y 6. Con la anterior modificación se entiende como superada esta observación.

2.2 Nivel inventivo

El nivel inventivo de una solicitud se encuentra definido en el Artículo 18 de la Decisión 486 y se establece cuando *“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”*.

Contrario a lo que plantea el Examinador, el documento del arte previo citado no afecta el nivel inventivo de la presente solicitud por cuanto no es posible derivar la presente invención a partir de las enseñanzas presentadas en el documento **D1**.

Se llama la atención a su Despacho en cuanto a que tanto el amitraz y la metaflumizona proporcionan dificultad en su formulación debido a los problemas de inestabilidad y solubilidad en varios solventes. Por favor, refiérase a la descripción página 1 donde se cita textualmente:

“El amitraz es, desafortunadamente, inestable químicamente en la presencia de disolventes que tengan un grupo hidroxilo reactivo tal como alcoholes, glicoles, agua y similares. Esta característica ha limitado el desarrollo de composiciones veterinarias que contienen amitraz, y especialmente aquellas que contienen amitraz y por lo menos un agente parasiticida adicional, debido a la combinación de la inestabilidad del amitraz en disolventes que contienen grupos hidroxilo”.

Una formulación ectoparasiticida tópica aceptable debe ser suficientemente fácil para aplicar, secar dentro de un periodo de tiempo razonable sin deteriorar la apariencia del animal (es decir, sin precipitación), ser suave en el pelaje del animal, sin irritar la piel de los animales y mantener su efectividad sobre el animal durante la actividad normal del animal, tal como la exposición al sol y al agua.

Además, esta debe ser capaz de aplicarse al animal en un volumen suficientemente pequeño de modo que se evite que el animal lama el área de aplicación.

De conformidad con lo anterior, es particularmente difícil lograr una composición efectiva que contenga metaflumizona y amitraz que muestre aceptabilidad cosmética sin irritación cuando se aplica sobre el animal.

Como lo demostró Sabnis et al., Vet. Parasitology 150, 196-202 a 198 (2007). Ver anexo.

El amitraz y la metaflumizona sorprendentemente ambos tienen un muy alto nivel de solubilidad en γ -hexalactona, que también tiene un efecto beneficioso de mejorar estabilidad en el almacenaje también como proporcionar el nivel correcto de esparcirse y penetración en la piel y el pelo sin dejar precipitación residual. En **D1** no se sugiere estos efectos, especialmente cuando se aplica en una formulación que comprende amitraz y metaflumizona.

Los solventes altamente polares que contienen grupos hidroxilo activos, incluyendo agua, alcoholes, glicoles y similares son empleados generalmente en aplicaciones tópicas porque son compatibles con el pelo y piel del animal y tienen la capacidad de disolver altas concentraciones de ingredientes activos.

Las composiciones de amitraz son deseables por su actividad contra garrapatas, incluyendo garrapatas resistentes a medicamentos. Hasta ahora, las composiciones veterinarias con otros activos han sido limitadas por la inestabilidad del amitraz en solventes con grupos hidroxilos (Talanta, Volumen 48, Issue 1,5, January 1999, pages 189-199).

De acuerdo con lo anterior, un desafío significativo que enfrentaron los inventores de la presente invención era formular composiciones que contienen altas cantidades de amitraz y metaflumizona en una composición efectiva, sin causar degradación de ninguno de los activos. Como se demostró en la tabla I, las composiciones reclamadas son estables y extremadamente eficaces por un largo periodo para el tratamiento tópico de infestación parasitaria en animales, un resultado que es inesperado a la luz del arte previo citado por su Despacho. Los resultados en la tabla 1 son efectivamente sorprendentes ya que demuestran que la composición de los inventores de la solicitud en estudio produce una eficacia del 86.5%-98.8% hasta un mes después del tratamiento. Esto significa que la dosis puede

llevarse a cabo en intervalos mensuales para el tratamiento efectivo contra pulgas y garrapatas.

En este orden de ideas, tenemos que la invención propuesta por el solicitante si cumple con los requisitos de patentabilidad, es decir, es novedosa tal como ya lo reconoció su Despacho y también presenta altura inventiva y de esta manera es susceptible de ostentar el título de patente de invención.

3. Solicitudes concedidas en otros países para esta misma invención.

Aunque compartimos la opinión de su Despacho en cuanto a que el otorgamiento de una patente equivalente a la presente solicitud Colombiana por otras Oficinas no obliga a su Despacho a actuar de la misma forma, no debe desatenderse el hecho de que la Oficina Peruana de patentes que se rige por la misma Decisión 486, haya reconocido la novedad y el nivel inventivo de la solicitud equivalente a la solicitud Colombiana No. 07.034171 y haya otorgado la patente No.5554.

De hecho, la concesión de las solicitudes correspondientes en otros países del mundo, resulta de gran importancia ya que este hecho ha sido considerado dentro del Artículo 46 de la Decisión 486, en el cual se establece que la Oficina Nacional competente podrá requerir informes de otras oficinas de propiedad industrial, para lo cual el solicitante podrá proporcionar copia de los resultados de exámenes de patentabilidad efectuados respecto de las solicitudes extranjeras correspondientes. En este sentido, de acuerdo con dicho Artículo 46, la Oficina Nacional competente podrá reconocer los resultados de los exámenes como suficientes para acreditar el cumplimiento de las condiciones de patentabilidad de la invención, como es el caso de las solicitudes concedidas en el extranjero.

En este sentido, debe tenerse en cuenta que la independencia que posee la *SIC* para juzgar la patentabilidad de una invención, se refiere más a las excepciones de patentabilidad que lógicamente pueden variar o a las causales de nulidad o cancelación, respecto de las cuales cada país puede tener su propio criterio. Sin embargo, tratándose de la apreciación de novedad y nivel inventivo, como el discutido en este caso, los cuales son requisitos *sine qua non* de toda patente, es evidente que los criterios de apreciación no varían mucho de legislación a legislación, y que una apreciación positiva acerca de la existencia de dichos

requisitos por parte varias Oficinas de Propiedad Industrial a nivel mundial, sí constituye un indicio más que razonable de que la solicitud es patentable.

En virtud de lo anterior, solicito que se acepten las razones expuestas en el presente documento y en consecuencia, se otorgue el privilegio de patente de invención para el invento que se tramita en este expediente.

Atentamente,


ÁLVARO CORREA ORDÓÑEZ
C.C.No.79.143.366 de Usaquén
T.P.20.281

Reivindicaciones

1. Una composición parasitocida caracterizada porque comprende de 10% a 30% peso/volumen de amitraz y de 10% al 30% peso/volumen de metaflumizona; en una mezcla de solventes que no contiene grupos hidroxilos que contiene N,N-dietil-m-toluamida y γ -hexalactona, y sulfóxido de dimetilo, eucaliptol, y acetato de 1-metoxi-2-propilo.
2. La composición de acuerdo con la reivindicación 1 en la que la mezcla del disolvente comprende de 2 a 30% peso/volumen de N,N-dietil-m-toluamida; de 2 a 30% peso/volumen de sulfóxido de dimetilo; de 2% a 30% peso/volumen de eucaliptol; de 2% a 40% peso/volumen de γ -hexalactona, y de 2% a 40% peso/volumen de acetato de 1-metoxi-2-propilo.
3. La composición de acuerdo con la reivindicación 1 en donde la mezcla de disolventes comprende de 5 a 25% peso/volumen de N,N-dietil-m-toluamida; 5 a 25% peso/volumen de sulfóxido de dimetilo; 5% a 25% peso/volumen de eucaliptol; 10% a 30% peso/volumen de γ -hexalactona, y 15% a 35% peso/volumen de acetato de 1-metoxi-2-propilo.
4. La composición de acuerdo con cualquiera de las reivindicaciones 1 a 3 que comprende 13% a 20% peso/volumen de amitraz y 13% a 20% peso/volumen de metaflumizona.
5. Un proceso para la preparación de una composición de acuerdo con la reivindicación 1, que comprende: mezclar N,N-dietil-m-toluamida y γ -hexalactona, con eucaliptol, dimetilsulfóxido y acetato de 1-metoxi-2-propilo para formar una mezcla de disolventes; y tratar a dicha mezcla

de disolventes con amitraz y metaflumizona para formar una solución homogénea, pasando dicha solución a través de un agente deshidratante sólido.

6. El proceso de acuerdo con la reivindicación 5, en el que el agente deshidratante sólido es tamizado molecularmente a 4Å.

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Topical formulations of metaflumizone plus amitraz to treat flea and tick infestations on dogs

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Abstract

A topical spot-on solution was developed for treating pets that contained of active ingredients metaflumizone and amitraz and intended for use as an ectoparasiticide. The formulation vehicle system was designed by balancing the following three attributes of various solvents: evaporation/drying, surface spreading, and percutaneous absorption. The solvents were selected by evaluating the solubilization capacity of individual solvents with respect to the above active ingredients. The evaporation rates of various solvent systems were then determined. The visual observations of the treatment sites was also performed a day after treating the dogs to understand the cosmetic effect of various solvent systems. The lead formulations dried off within a day after application with no noticeable residue at the treatment site, while others produced appreciable powdery residue or a large wet and oily spot. The stability of the lead formulations was also evaluated over 2 years to demonstrate a 2-year shelf life of this product.

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Keywords: Metaflumizone; Amitraz; Ectoparasiticide; ProMeris®; ProMeris Duo®; Fleas; Ticks

1. Introduction

In the past the ability to develop topical dosage forms, often creams and ointments, has been described as art. With the availability of multifunctional adjuvants and sophisticated machines, the technology has advanced significantly in recent years. Topical formulations present unique and challenging demands in that (1) they should have both chemical and physical stability for at least 2 years, (2) have components that are nonirritating, non-sensitizing and non-allergenic, (3) are cosmetically acceptable and preferably cosmetically elegant, and (4) are efficacious because of their ability to deliver therapeutic levels of drug over a

defined period of time. The realization that changes in vehicles/adjuvants can significantly change efficacy has permanently altered the way pharmaceutical scientists view topical formulations. The formulator is required to design dosage forms that target follicles or other specific skin regions, select vehicles that optimize drug–skin interactions or vehicles that release drugs for sustained periods of time (Osborne and Amann, 1990).

Since percutaneous absorption is common to both topical and transdermal products, there has been a tendency to view this event as being of comparable significance in both delivery modalities. For transdermal products, an optimal drug flux across the skin without appreciable drug build-up in the skin is ideal, while for topical products an optimal drug build-up in the skin with little or no drug flux through the skin is most desirable. Topical formulations usually contain several excipients such as permeation enhancers,

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spreading agents, etc. that partition into the skin in accordance with their physicochemical properties. Certain excipients change the integrity of the stratum corneum (Cornwell and Barry, 1993). When this occurs, the ease with which the active ingredients diffuse through this tissue is affected. Some components evaporate during the course of delivery, systematically increasing the drug concentration in the remaining vehicle/solvent. This means that localization within skin depends upon the unique properties of the formulations. Depending upon the specific formulation used, a high degree of drug accumulation in the skin may be achieved even at very low flux values (Shah et al., 1992).

This report addresses the challenges involved in the development of an efficacious topical spot-on formulation containing a novel active ingredient, metaflumizone, for use as an ectoparasiticide for domestic animals. Spot-on formulations are typically small volume liquids that are applied in one or two spots on the back of the animal at a location that cannot be licked off by the animal's tongue (Klink et al., 1998). Metaflumizone (IUPAC hydrazinecarboxamide, 2-[2-(4-cyanophenyl)-1-[3-(trifluoromethyl)phenyl]ethylidene]-N-[4-(trifluoromethoxy)phenyl]) is an excellent neuronal sodium channel antagonist. A neuronal sodium channel antagonist functions by preventing the ability of a neuron cell to transfer sodium ions across the cell membrane. A neuron cell thus affected is not capable of propagating a neuronal impulse, resulting in paralysis, and ultimately mortality in the target pest (Heaney et al., 2004). Metaflumizone is highly efficacious against fleas (e.g. *Ctenocephalides* sp.) and can also be used in combination with an acaricide, such as amitraz, for treatment against ticks (e.g., *Rhipicephalus sanguineus*, *Dermacentor variabilis*, *Amblyomma americanum*, *Ixodes scapularis*, etc.) (Heaney et al., 2004; Rugg et al., 2007).

The objective of the present study was to develop a spot-on solution for a metaflumizone plus amitraz combination for dogs at a dose of 20 mg/kg of each active ingredient (ProMeris®/ProMeris Duo® for Dogs, Fort Dodge Animal Health, Overland Park, KS). The formulation vehicle system was designed by balancing three attributes: (1) evaporation/drying, (2) surface spreading, and (3) percutaneous absorption. Since spot-on formulations are applied in low dose volumes, good solubility of the active ingredients in each of the solvents of the formulation was imperative. Therefore, all potential vehicles were tested for solubilization capacity followed by drying and spreading characteristics.

2. Materials and methods

2.1. Saturation solubility study

To evaluate the solubility of each active ingredient in each of the potential solvents, several binary mixtures of one active ingredient and one vehicle were prepared in 20 ml scintillation vials. This was done by measuring approximately 4 g of the active ingredient and adding 6 g of the following solvents to the vials: 2-(2-butoxyethoxy)ethyl acetate, 2-butoxyethyl acetate, 2-phenoxyethanol, benzyl alcohol, benzyl benzoate, cineole (eucalyptol), diethylene glycol monoethyl ether, dimethyl sulfoxide, dipropylene glycol methyl ether, fatty alcohol ethoxylate surfactant, γ -hexalactone, heptyl acetate, isopropyl myristate, isopropylidene glycerol, *N,N*-diethyl-*m*-toluamide, oleic acid, polyethylene glycol 400, propylene glycol diacetate, and propylene glycol methyl ether acetate. The sample mixtures were then shaken on a Cole Palmer orbital shaker (Series 51300) at 200 rpm for 48 h at 22–25 °C. The system was designed to ensure that excess solid remained at all times in the samples to provide a saturated solution. The supernatant (approximately 1 ml) was filtered using a 0.8 μ m nylon filter disc and assayed for active ingredient concentration by methods described elsewhere (unpublished method).

2.2. Effect of co-solvent concentrations on evaporation rate

The experimental formulations were set to contain 15% (w/v) of each of the active ingredients. The ideal formulation is a balance between the properties of solubilization, depot in the skin, evaporation/drying and spreading on the surface of the skin. Since the actives were required to depot in the skin rather than penetrate through the skin, the level of the skin partitioning component was minimized to 20% (w/v) of the formulation total. The levels of the solubilizing component, the surface spreading component and the evaporation/drying component were optimized by performing a design of experiments exercise (Anderson and Whitcomb, 1998). The level of the solubilizing but nonevaporating and nonspreading component was varied between 10 and 25% (w/v). The spreading component was varied between 0 and 30% (w/v) and the volatile component was varied between 10 and 25% (w/v). Sixteen mixtures were prepared in 20 ml scintillation vials wherein the concentrations of these components were varied such that the extreme limits as well as the central regions of the three components could be

studied. The evaporation rates were determined by studying the change in weight due to evaporation of the solutions over a 24-h period at 25 °C. A surface contour plot of the evaporation rate versus concentrations of various mixture components was thus obtained.

2.3. Post-application cosmetic evaluation of the formulations

Since the formulations were designed for domestic pets, it was essential to have (1) quick drying of the dose (within 1 day) after application, and (2) no drug residue at the site of application. However, satisfying these conditions became a complex issue as the rapidly evaporating formulations tended to leave noticeable drug residue on the hair coat and the solvent systems that did not show any drug precipitate tended to remain wet for an extended period of time. The experiments were focused on striking a balance between the rates of evaporation, spreading and permeation of the mixture by sequentially changing the concentrations of the components responsible for these attributes. Three beagle or mix-breed dogs were selected per treatment group and housed indoors in individual pens. The experimental spot-on formulations were applied on the back of the neck as one spot at a rate of 1.34 ml/10 kg body weight. Visual observations of the application sites were made 4, 24, and 48 h post-application. The final formulation was optimized so that no drug precipitate or wet spot was observed at the application site at 24 h.

2.4. Stability evaluation of the optimized product

The stability of the optimized product was studied to determine its shelf life. The formulation was packaged in amber glass vials with Teflon[®] faced rubber stoppers and stored at the 25 °C and 60% relative humidity (RH) for up to 24 months. The samples were periodically tested for the active ingredient concentrations by established assay methods (Fort Dodge Animal Health company documents).

3. Results

3.1. Saturation solubility study

A number of solvents were evaluated with respect to the solubilization properties (Table 1). The solubility of the active ingredients varied significantly amongst various solvents. The solvents that dissolved high levels of both the actives were deemed suitable for further evaluation for formulation development.

Table 1
Saturation solubility results of metaflumizone and amitraz in various solvents^a

Solvent	Metaflumizone solubility (% w/w)	Amitraz solubility (% w/w)
2-(2-Butoxyethoxy)ethyl acetate	9.68	23.2
2-Butoxyethyl acetate	11.20	26.6
2-Phenoxyethanol	1.08	14.1
Benzyl alcohol	1.57	20.7
Benzyl benzoate	1.58	24.4
Cineole (Eucalyptol)	0.47	24.7
Diethylene glycol monoethyl ether	8.57	11.1
Dimethyl sulfoxide	37.13	14.8
Dipropylene glycol methyl ether	10.85	21.4
Fatty alcohol ethoxylate surfactant	5.28	13.3
γ-Hexalactone	24.18	31.1
Heptyl acetate	11.38	28.5
Isopropyl myristate	0.44	16.2
Isopropylidene glycerol	1.43	8.29
N,N-Diethyl-m-toluamide	31.22	33.9
Oleic acid	0.73	7.53
Polyethylene glycol 400	1.54	5.10
Propylene glycol diacetate	12.64	15.4
Propylene glycol methyl ether acetate	18.26	25.4

^a The values represent means of two individual observations for each solvent and active ingredient.

3.2. Co-solvent concentration effect on evaporation rate

All the mixtures were prepared such that vehicles composed of 70 parts of the mixture and the two actives contributed to 15 parts each. The different mixtures used in this experiment and the resultant evaporation rates are presented in Table 2. A response surface plot was constructed of the evaporation rate versus concentration of each of the vehicle component (Fig. 1).

As seen from Fig. 1, changes in the surface spreading component had considerable effect on the overall evaporation rate of the mixture, while altering the relative concentrations of the solubilizing component and the evaporating component did not have as much impact on the overall rates of evaporation. The component ratio in the formulation for the commercial product was chosen as follows: a plateau region, depicted by a dotted ellipse in Fig. 1. The area within the ellipse defines where (1) the evaporation rate was sufficiently high, and (2) a moderate change in the mixture's composition did not dramatically alter the resultant mixture's evaporation rate. Various solvent ratios within this region were attempted and cosmetic evaluations were performed. The results are described in the next section.

Table 2
Design matrix for optimization of vehicle system and resultant evaporation rates

Absorption enhancer (% w/w)	Solubilizing component (% w/w)	Surface spreading component (% w/w)	Evaporating component (% w/w)	Evaporation rate (mg/h cm ²)
20	10	30	10	1.139
20	10	25	15	1.050
20	10	20	20	0.994
20	10	15	25	0.960
20	15	25	10	1.118
20	15	20	15	0.963
20	15	15	20	0.899
20	15	10	25	1.139
20	20	20	10	0.990
20	20	15	15	0.716
20	20	10	20	0.602
20	20	5	25	0.343
20	25	15	10	0.758
20	25	10	15	0.668
20	25	5	20	0.577
20	25	0	25	0.546

3.3. Post-application cosmetic evaluation of the formulations

The concentrations of (1) solubilizing component, (2) evaporating/drying component, and (3) surface spreading component were sequentially changed within the boundaries identified by the dotted circle in evaporation rate experiment (Fig. 1) discussed above. The cosmetic appearance 24 h after application on the animal's back was then evaluated. Photos of some representative formulations are presented in Figs. 2–7. Fig. 2 illustrates the effect of including high levels of the solubilizing component. A distinct oily patch was visible even after 24 h post-application. In Fig. 3, a white powdery residue of the active ingredients was

observed on the hair shafts at the site of application as a result of high levels of evaporating components. In Fig. 4, the effect of addition of high level of surface spreading component is illustrated. Here the product spread over a larger area on the back of the animal and was still wet after a 24 h. Some precipitate was also observed on the hair shafts. All the above conditions would render the product unacceptable by the consumer. Upon optimization of the levels of different components in the formulation, the change in the resultant cosmetic effect is evident in Figs. 5–7. These photos show the effect on dogs with different colored haircoat. The product appears to have essentially dried within 24 h with no visible product residue or precipitate.

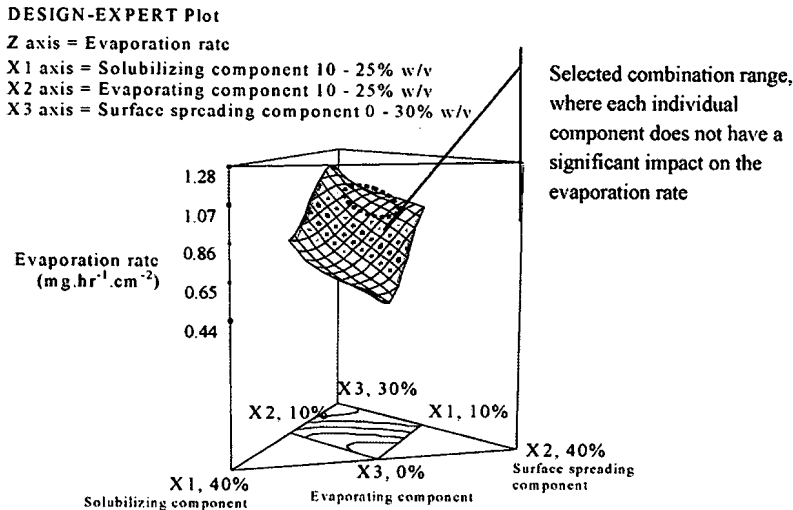


Fig. 1. The surface contour plot of evaporation rates of various solvent systems.



Fig. 2. The cosmetic effect of inclusion of high level of the solubilizing component.

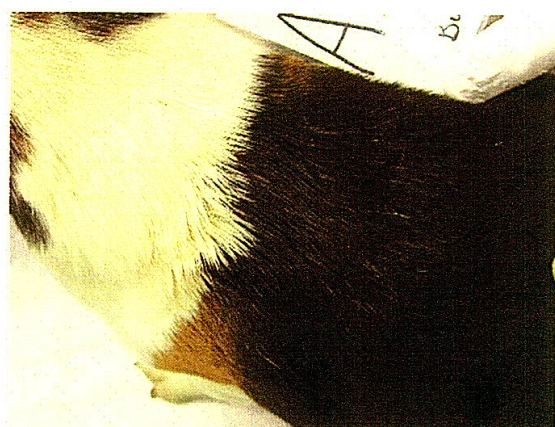


Fig. 5. The cosmetic evaluation of the optimized formulation—example 1.



Fig. 3. The cosmetic effect of inclusion of high level of the evaporating component.



Fig. 6. The cosmetic evaluation of the optimized formulation—example 2.



Fig. 4. The cosmetic effect of inclusion of high level of the surface-spreading component.



Fig. 7. The cosmetic evaluation of the optimized formulation—example 3.

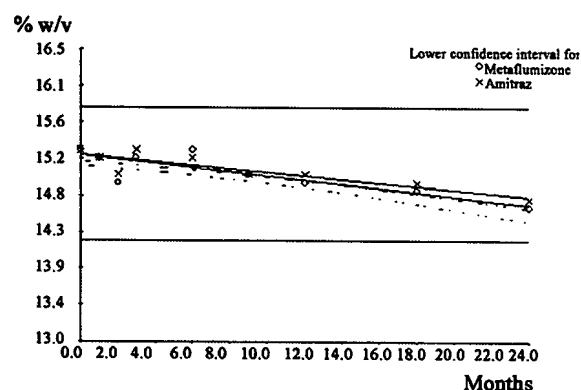


Fig. 8. Stability of metaflumizone and amitraz spot-on formulation packaged in amber glass vials and stored at 25 °C and 60% RH.

3.4. Stability evaluation of the optimized formulation

Since the typical shelf life of a topical parasiticide product is 2 years (Rathbone et al., 2000), the shelf life stability of the optimized formulation was studied for 2 years. The results (Fig. 8) show that at least 95% of each active ingredient remains at the end of 2 years based on the lower 95% confidence intervals obtained from the regression analysis ($P < 0.25$). Therefore, a 2-year shelf life is appropriate for this product.

4. Discussion

The criteria for selecting an appropriate formulation for a topical flea treatment for dogs and cats are good solubility of the active ingredients, good adhesion to the skin, good spreading properties, good local, and systemic tolerance, stability, and compliance within regulatory standards (Krämer and Mencke, 2001). The saturation solubility experiments with a variety of solvents allowed the formulators to choose the solvents that dissolved both the actives at high levels. At 30% w/v (i.e., 15% of each of the active ingredients), the drug loading in the formulation was quite high but this allowed the product to be applied at low volumes on the animals. This makes the formulation more appealing and cosmetically elegant. Our experiments involving higher concentrations of active ingredients did not yield better results. These observations corroborate well with studies involving other actives. For example, Krämer and Mencke (2001) have reported that a 10% solution of imidacloprid was more effective than a 20% or a 30% solution applied at the same dose level. The 10% solution spread more effectively over the skin than the concentrated solutions thus providing better insecticidal activity.

After topical application in dogs and cats, the drug can be transported into the haircoat, the skin itself and the circulation. Krämer and Mencke (2001) have identified the skin surface as the effective carrier of the active ingredient after dermal application and the one producing the ectoparasitocidal effect of imidacloprid. Furthermore, topical ectoparasitocidal products have been shown to distribute all over the body of dogs and cats within as little as 12 h after application. Similar results were obtained for this formulation as well, wherein after application of the product between the shoulder blades of the dogs, the product was detectable at the base of the tail within a day and was detectable at significant levels even after 28 days post-application (DeLay et al., 2007). The other likely channel of distribution for the active ingredients is the skin. Although the transdermal uptake of the actives into the bloodstream was found to be negligible for this formulation of metaflumizone plus amitraz (DeLay et al., 2007), the possibility of distribution and depot of the actives in the stratum corneum and deeper in the epidermis cannot be eliminated. Low angle X-ray diffraction studies have revealed the lipid–protein compartmentalization in the stratum corneum. The intercellular spaces in the epidermal region are composed of lipid bilayers (Friberg et al., 1990). The lipid composition in dog has been reported to be primarily nonpolar in nature, specifically sterol esters (42%), wax diesters (32%), and free sterols (9%), while polar lipids are about 7% (Sharaf et al., 1977). As a result, the transport of a lipophilic moiety parallel to the lipid bilayers is fast, the same magnitude as would be seen in a liquid with minimal resistance (Friberg et al., 1990). This phenomenon can be further exploited by utilizing organic solvents that are miscible with lipids of the skin. The vehicle system for this metaflumizone plus amitraz formulation was selected to facilitate the distribution of active ingredients across the skin. The distribution and depot of the active ingredients in the stratum corneum and epidermal layers of the skin has two distinct advantages: (1) prolongation of efficacy and (2) minimal loss of efficacy due to bathing or because of rain. The selection of a balanced vehicle system further prevented loss of actives by minimizing their precipitation on the hair coat. As a result most of the active ingredients were partitioned into the skin layers producing maximum therapeutic effect. The photographs of the application sites treated with the optimized formulation (Figs. 5–7) showed negligible drug residue. It was further demonstrated that the vehicles selected for this formulation also provided

adequate stability for the product, which is an essential aspect of an efficacious product.

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