

Can methylisothiazolinone be patch tested in petrolatum?

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doi:10.1111/cod.12188

Key words: allergic contact dermatitis; chemical investigations; contact allergy; high-performance liquid chromatography; patch test preparations; preservative.

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Conflicts of interest: The authors declare no conflict of interests.

In 2005, just before the introduction of methylisothiazolinone (MI) as a preservative in cosmetic products, it was decided within the European Environmental and Contact Dermatitis Research Group to patch test with MI as an additional test preparation in each centre's baseline series, to try to find the most optimal test concentration for this preservative (1). When

discussing which patch test preparation to use, some centres asked whether it would be possible to use pet. From earlier experience conveyed by Rohm and Haas (Philadelphia, PA, USA), the company providing MI at the time, we had been informed that pet. was impossible to use as a vehicle for MI, but to further substantiate this we conducted a chemical investigation to determine whether MI could be evenly dispersed in pet. at the concentrations that we aimed to use, namely 1000, 1500 and 2000 ppm MI (1).

Case Report

Methanol was obtained from J. T. Baker (Mallinckrodt Baker B.V., Deventer, The Netherlands). The water was Millipore water. High-performance liquid chromatography (HPLC)-grade heptane was purchased from Fisher Scientific (Loughborough, UK). MI was obtained from Neolone™ 950 (9.5% MI), which contains only water and MI, kindly supplied by Rohm and Haas.

From a stock solution of MI, three pet. preparations of MI were made, weighing ~ 5 g each, containing 1000, 1500 and 2000 ppm MI, respectively. Each mixture of MI and pet. was stirred manually for 5 min before being placed into a syringe. From each syringe, 10 samples of ~ 0.5 g each were drawn. Before HPLC analysis, an extraction procedure at room temperature was performed. Each sample was accurately weighed, placed into a test tube, and diluted with 10 ml of 99% heptane. This mixture was shaken vigorously until the pet. had dissolved. Then, 10 ml of water was added, and the tube was shaken again. Two phases were then seen, the aqueous phase being underneath the heptane phase. From the aqueous phase in the tube, a sample was drawn and injected into the column.

Five pet. preparations of MI were made. Each preparation was prepared by accurately weighing ~ 26 mg of Neolone™ 950, placing it into a 50-ml test tube, and then adding ~ 2 g pet. (accurately weighed). Fifteen millilitres of heptane was added to the tube, which was then shaken vigorously until the pet. had dissolved. Three 15-ml volumes of water were added, the tube was again shaken, and the water phase was removed and placed into a 50-ml beaker, which was filled with water. The solution was diluted 20-fold with the eluate methanol/water (30/70 vol/vol) before being injected into the HPLC system.

For isolation of MI, a ThermoFinnigan system was used, consisting of a P4000 quaternary pump, an ultraviolet 6000 diode array detector, an AS 3000 autoinjector, an SN4000F control module, and software controlled by CHROMQUEST 4.1 and monitored by SPECTRAL ANALYSIS

FOR CHROMQUEST (ThermoFinnigan, San José, CA, USA). The injection volume was 20 µl. The column used was a Kromasil (4.6 mm internal diameter × 250 mm, 5 µm, 100 Å; EKA Nobel, Bohus, Sweden). The detector scanned the eluate in the range 200–360 nm, and chromatograms recorded at 280 nm were used for the detection and measurements. The elution was isocratic with methanol/water (30/70 vol/vol) and the flow rate was 0.8 ml/min. Aqueous reference standard preparations of Neolone™ were made containing 0.00025%, 0.0005% and 0.001% wt/vol Neolone™, respectively.

The recovery of a known amount of MI in pet. showed the average recovery to be 96.5% (range 94.2–100%) (Table 1).

In Table 2, the percentage yield from each preparation is shown. For the MI 1000 ppm preparation, the yield varied between 27.8% and 110.4%, with a mean ppm value of 895.5; for 1500 ppm, the variation was between 88.3% and 256.8%, with a mean ppm value of 1736; and for 2000 ppm, the variation was between 93.9%

Table 1. The recovery of Neolone™ 950 as percentage of added Neolone™

Amount of Neolone™ 950 added (mg)	Amount of pet. added (g)	Percentage of Neolone™ 950 in pet. preparation	Recovery (%)
24.6	2.1657	0.1158	97.7
26.3	2.3584	0.1059	94.9
26.2	2.6648	0.0934	95.9
26.6	1.228	0.2058	94.2
23.3	1.256	0.1762	100

Table 2. The yield of methylisothiazolinone (MI) [ppm (% of added MI)] in each pet. preparation in 10 samples from each preparation

1000 ppm	MI preparation	
	1500 ppm	2000 ppm
1104 (110.4)	1460 (97.3)	2189 (109.4)
1010 (101.0)	1472 (98.1)	2180 (109.0)
975 (97.5)	1467 (97.8)	2168 (108.4)
1000 (100)	1417 (94.5)	1878 (93.9)
1003 (100.3)	1325 (88.3)	2167 (108.3)
828 (82.8)	1634 (108.9)	2147 (107.3)
278 (27.8)	1674 (111.6)	2170 (108.5)
848 (84.8)	1480 (98.7)	2172 (108.6)
943 (94.3)	1578 (105.2)	2178 (108.9)
966 (96.6)	3853 (256.8)	2168 (108.4)
MV 895.5 ppm (89.6)	MV 1736 ppm (115.7)	MV 2142 ppm (107.1)
SD 231.11 ppm	750.95 ppm	93.30 ppm
CV 25.81	43.25	4.36

CV, coefficient of variation (%); MV, mean value; SD, standard deviation.

and 109.4%, with a mean ppm value of 2142. The standard deviations were 231.11, 750.95, and 93.29, respectively, and the intra-assay coefficients of variation for the analysis of MI in the respective pet. preparations (1000, 1500 and 2000 ppm MI) were 25.81%, 43.25%, and 4.36%, respectively.

Discussion

In this chemical investigation, we set out to examine the variability in the yield of MI in three pet. preparations loaded with 1000, 1500 and 2000 ppm MI. To achieve this, we had to perform a recovery analysis, as we had

performed an extraction procedure. The recovery analysis showed high values. Concerning the yield of MI, had we found low variability, one of the prerequisites for an adequate test preparation would have been fulfilled. However, the standard deviations were high and the variation between yields was high, with coefficients of variation of 25.81 and 43.25, indicating that the MI molecules were not evenly enough distributed in the pet. In the information from Rohm and Haas, it was stated that pet. is not an adequate vehicle for MI, and we demonstrated that this was the case. Consequently, MI should not be patch tested in pet., as has been done in some centres, as presented in a recent paper reviewing patch testing with MI (2).

References

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