

Investigation of the homogeneity of methacrylate allergens in commercially available patch test preparations

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Summary

Background. The homogeneity of methacrylates in commercial patch test preparations has not yet been investigated. Inhomogeneous patch test preparations may give rise to false-negative or false-positive patch test results in patients suspected of having methacrylate allergy.

Objectives. To investigate the homogeneity of methacrylates in commercial patch test preparations.

Methods. Fresh commercial patch test preparations of methyl methacrylate (MMA) and 2-hydroxyethyl methacrylate (2-HEMA) from three test material suppliers in Europe were analysed quantitatively by means of normal-phase high-performance liquid chromatography.

Results. The initial concentration of MMA in all six patch test preparations was lower than stated on the label, whereas four of six patch test preparations of 2-HEMA were in accordance with the stated concentrations. The concentration of MMA increased markedly from the top segment close to the tip of the syringe to the bottom segment adjacent to the piston in four syringes (3–6). In contrast, syringes with 2-HEMA maintained a constant concentration throughout the test preparation, apart from two syringes (11 and 12).

Conclusion. Variations in concentration and heterogeneous distribution of MMA and 2-HEMA in patch test preparations may be an additional cause of variation in patch test results, besides other technical details and reading.

Key words: commercial patch test preparations; homogeneity; HPLC; methacrylates; volatile allergens.

Patch test technique is a crucial source of variation in the diagnosis of contact allergy. It includes all technical aspects, such as the test application device,

the precise dose of test material, and the quality of the test preparation. Diagnostic patch test technique is under continuous development and refinement in order to optimize it for routine use. Several aspects, such as the choice of vehicle, the stability and purity of allergens, the allergen concentrations, and the distribution of allergen throughout the vehicle, may all have a significant impact on the outcome of the patch test (1). Recently, attention has been focused on the stability of volatile allergens in patch test preparations. There is a decrease in concentration when pet. samples of acrylates/methacrylates are stored in patch test chambers at room temperature and in the refrigerator. In addition, the initial measured concentration in fresh commercial patch test preparations is lower than stated by the

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suppliers (2–4). Another technical source of variation may be the homogeneity of the distribution of contact allergens in commercial patch test preparations. The objective of the present study was therefore to investigate the homogeneity of two volatile methacrylates in patch test preparations from three different suppliers.

Materials and Methods

Reagents

High-performance liquid chromatography (HPLC)-grade solvents, heptane and ethanol, were purchased from Rathburn Chemicals (Walkerburn, UK), and butanol was acquired in analytical-grade quality from BDH Prolabo, VWR (Herlev, Denmark). Analytical standards of methyl methacrylate (MMA) and 2-hydroxyethyl methacrylate (2-HEMA) were obtained from Sigma-Aldrich (Steinheim, Germany). Fresh commercial patch test preparations were weighed on an analytical scale with a precision of 0.01 mg (XS 205 Dual Range; Mettler Toledo, Glostrup, Denmark). All solvents and test materials were filtered through 0.45- μ m organic nylon filters (Frisenette Aps, Knebel, Denmark), and stored briefly in Agilent Technologies (Santa Clara, CA, USA) screwcap vials prior to HPLC analysis.

HPLC apparatus and chromatographic conditions

The chromatographic analysis was performed with an Agilent Technologies HPLC 1200 series equipped with a G1315D diode array detector (DAD), a G1311A quaternary pump, a G1329A automatic liquid sampler, a G1316A thermostatted column compartment, and a G1322A vacuum degasser (Agilent Technologies). The DAD was operated between 190 and 400 nm, and the methacrylates were monitored at 215 nm. A normal-phase Luna 3 μ m Silica (2) 100 Å column (150 $\times$ 3 mm internal diameter; particle size 3.0 μ m; Phenomenex, Torrance, CA, USA) and a silica precolumn (4 $\times$ 3 mm; Phenomenex) were used for separation and column protection, respectively. The column temperature was set at 25°C. The chromatographic data were collected and processed with Agilent CHEMSTATION software (Revision B.04.01, Service Pack 1). The mobile phase consisted of 99% heptane + 1% butanol (MMA) and 90% heptane + 10% ethanol (2-HEMA). The HPLC settings were as follows: flow rate of the mobile phase, 0.5 ml/min; injection volume, 20 μ l; run time, 10 min; and post-time, 1 min. The presented HPLC method is based on a similar HPLC method developed by Goon et al., and has been used in previous studies (3, 4).

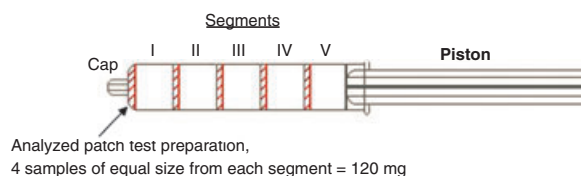


Fig. 1. Schematic illustration of a patch test syringe.

The repeatability of the employed HPLC method was determined by repeated analysis ($n = 7$) of three standard solutions, all of which were part of the actual calibration curve. The method has a relative standard deviation of $< 0.3\%$ for both methacrylate allergens.

Patch test preparations

In total, 12 fresh commercial patch test preparations were obtained from three of the major test suppliers in Europe. The patch test preparations, denoted 1–12, were labelled as containing either 1% or 2% w/w methacrylate allergen in pet. Each patch test preparation consisted of 5 ml of pet. mixed with the allergen, and was kept in the original capped test syringe prior to the analytical work. The content of each syringe was divided into five segments of equal size. Four consecutive samples were taken from the initial part of each segment (Fig. 1). Prior to sampling, no test preparation was squeezed out of the syringes.

The patch test preparations were stored according to the recommendations given by the patch test suppliers. Two of the suppliers recommended that the patch test preparations should be stored at temperatures between 2 and 8°C, whereas the third supplier's recommendation was: 'Store dark at 5–8°C'.

Sample preparation of the patch test preparations

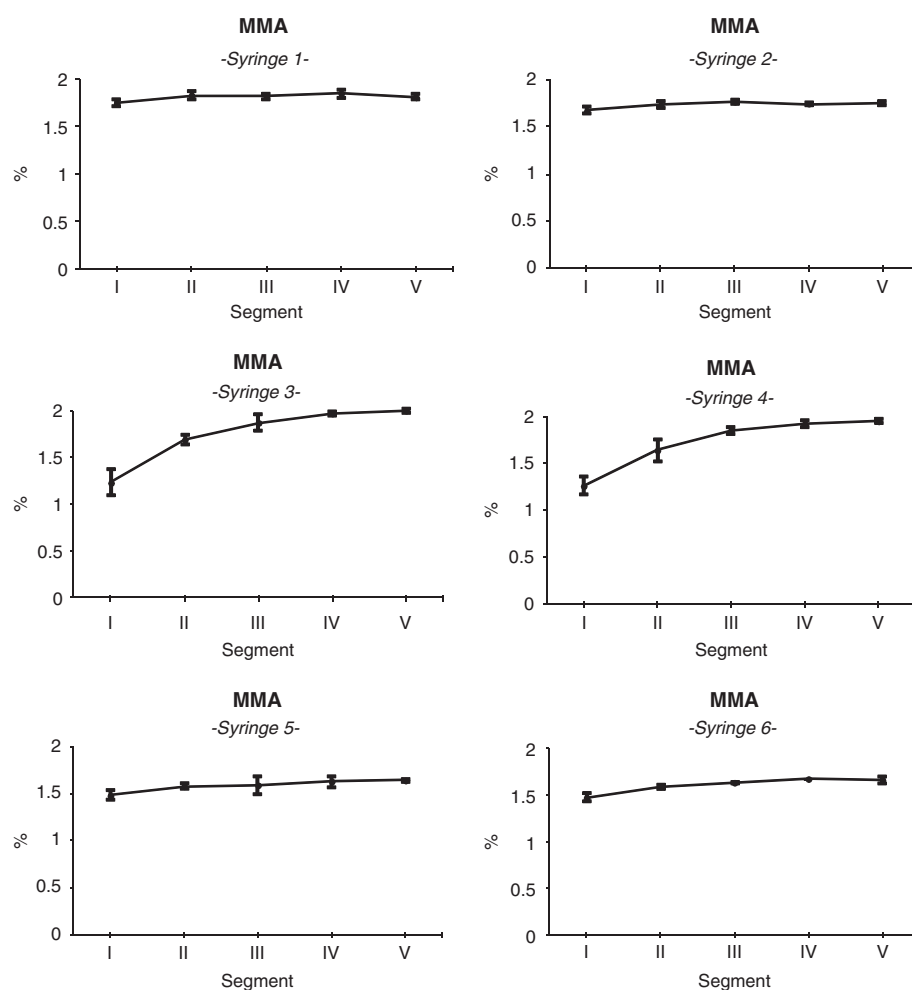
Approximately 30 mg of patch test preparation was weighed, placed in 25-ml volumetric flasks, and diluted in either 99% heptane + 1% butanol (MMA) or 99% heptane + 1% ethanol (2-HEMA). The samples were shaken until the test preparations were completely dissolved in the solvent mixture. Finally, ~ 0.5 ml of solution was filtered through organic nylon filters, placed in 1-ml vials, and analyzed.

Results

The ratio of measured to stated methacrylate concentration was calculated, and varied from 0.62 to 0.88 for MMA and from 0.71 to 1.08 for 2-HEMA (Table 1). Thus, the initial concentration of MMA in all of the commercial patch test preparations was lower than stated on the label,

Table 1. Summary of patch test preparations from three major test suppliers (analyzed in May–June 2012)

Syringe no. (supplier)	Stated concentration (%w/w)	Measured concentration (%w/w) (range)	Ratio of measured to stated concentration	Expiry date
Methyl methacrylate				
1 (A)	2	1.75 (1.70–1.78)	0.88	November 2013
2 (A)	2	1.67 (1.64–1.72)	0.84	November 2013
3 (B)	2	1.23 (1.04–1.34)	0.62	May 2013
4 (B)	2	1.26 (1.13–1.34)	0.63	May 2013
5 (C)	2	1.48 (1.41–1.54)	0.74	March 2014
6 (C)	2	1.47 (1.41–1.51)	0.74	March 2014
2-Hydroxyethyl methacrylate				
7 (A)	2	2.16 (1.74–2.32)	1.08	August 2013
8 (A)	2	1.97 (1.78–2.2)	0.99	August 2013
9 (B)	1	1.01 (0.96–1.09)	1.01	September 2013
10 (B)	1	0.95 (0.86–1.02)	0.95	September 2013
11 (C)	2	1.42 (0.76–1.93)	0.71	January 2013
12 (C)	2	1.75 (1.67–1.86)	0.88	January 2013

**Fig. 2.** The variation in distribution of methyl methacrylate (MMA) throughout petrolatum in patch test preparations from three major patch test suppliers in Europe (mean $\pm$ standard deviation).

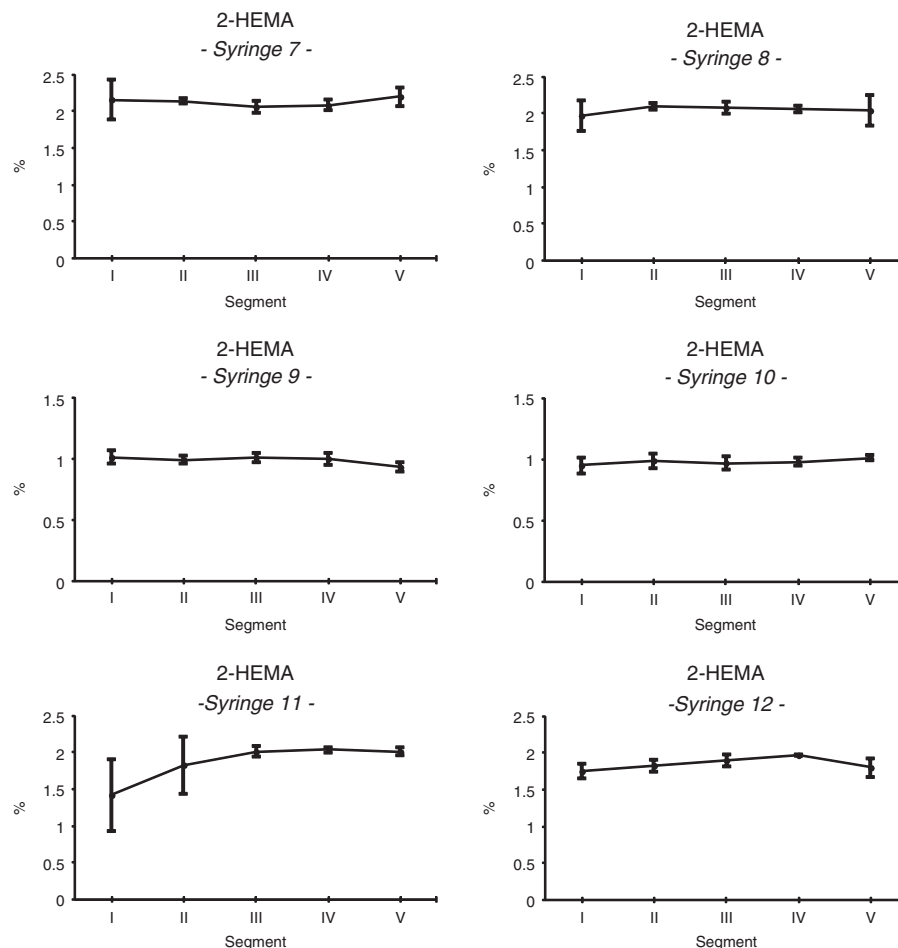
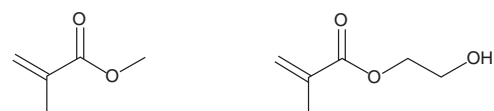


Fig. 3. The variation in distribution of 2-hydroxyethyl methacrylate (2-HEMA) throughout petrolatum in patch test preparations from three major patch test suppliers in Europe (mean ± standard deviation).

whereas all but two of the 2-HEMA preparations were in accordance with the stated concentrations. Furthermore, the variation in concentration found between samples from the first segment of the patch test preparations was large. Large standard deviations were also found in several segments for 2-HEMA in syringes 7, 8, 11, and 12 and the same applied for MMA in syringes 3–5. This shows that there is a variation in concentration between samples in each segment (Figs. 2 and 3).

In general, MMA showed a marked increase in concentration from segment I to segment V in syringes 3–6. 2-HEMA, on the other hand, maintained a constant concentration in all patch test preparations, with the exception of syringes 11 and 12. Overall, six of 12 patch test preparations showed a large variation in allergen distribution throughout pet. Consequently, the variation in dose per unit area between 20-mg pet. samples administered in small (8 mm) Finn Chambers[®] is significant for MMA. For example, the dose per unit area for samples in segment I and segment V from syringe 3 varied from 0.41 to 0.80 mg/cm² (Fig. 2).



Methyl methacrylate (MMA)
(CAS no. 80-62-6; vapour pressure 40 mmHg at 25 °C)

2-Hydroxyethyl methacrylate (2-HEMA)
(CAS no. 868-77-9; vapour pressure 0.01 mmHg at 25 °C)

Fig. 4. Chemical structures and physical characteristics of the methacrylates investigated in the present study.

Discussion

Several studies have described a lack of stability of volatile contact allergens in patch test chambers and in syringes under various conditions (2–11). This study shows that there is also a heterogeneous distribution of MMA and 2-HEMA in pet. in fresh commercial patch test preparations. The heterogeneous distribution of these methacrylates, together with the lack of stability, should be taken into consideration whenever a possible MMA or 2-HEMA allergy is investigated.

A possible contributory factor explaining the uneven distribution of MMA and 2-HEMA in pet. could be that the small liquid MMA and 2-HEMA droplets in pet. coalesce over time to form larger vesicles (12). Another concern is the analytical method: the semi-solid nature of pet. makes it difficult to ensure a homogeneous distribution of MMA and 2-HEMA in pet. test preparations. In addition, the volatility of MMA and 2-HEMA makes it difficult to control their concentrations in pet., owing to emission from this matrix. Hence, studies of recovery of MMA and 2-HEMA from pet. are subject to a high level of uncertainty, and therefore may not reflect the true recovery of these methacrylates from pet. test preparations. Consequently, studies of recovery of MMA and 2-HEMA from pet. test preparations were not performed.

In several stability studies, including the present investigation, a variation of $\pm 20\%$ of the stated concentration in patch test preparations has been defined as acceptable (2–5, 7–10). Of the 12 fresh preparations in this study, only seven fulfilled this requirement. The lack of stability and inhomogeneity of volatile contact allergens in pet.-based preparations is therefore of concern. Our data indicate that MMA preparations are generally more inhomogeneous and unstable than 2-HEMA preparations.

The most likely explanation for the lack of stability of methacrylates is evaporation, owing to their high vapour pressures (2–4) (Fig. 4). As the measured concentration was also lower in segments adjacent to the piston, other factors may be involved, such as chemical degradation (oxidation or hydrolysis), diffusion into the plastic of the syringe, suppliers' compounding errors, or spontaneous polymerization (2, 3). The last of these has been suggested

to occur when acrylic monomers dissolved in acetone have been dispersed in an aluminium chamber (13, 14). In these reports, it was stated that the aluminium oxide or an aluminium impurity, present on the aluminium surface, could possibly act as a catalyst and enhance the polymerization of the monomer. However, the pet. vehicle is considered to prevent polymerization of acrylate/methacrylate monomers.

In practice, it is neither customary nor recommended to squeeze out 100–200 mg of test preparation prior to sampling from commercial patch test preparations, although this has been described in a few experimental acrylate/methacrylate studies (2, 3). This was presumably done to prevent the use of a test preparation with a low allergen concentration. We suspect that the concentration of MMA and 2-HEMA will continue to decrease over time in the distal part of the pet. preparation, owing to evaporation during use of the syringe for testing eczema patients.

Stable, homogeneously distributed MMA and 2-HEMA test materials in pet. are obviously problematic to make. Therefore, there is a potential risk of variation in patch test results being caused by MMA and 2-HEMA patch test preparations having heterogeneous distributions of the test chemical.

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References

- De Groot A C. *Patch Testing. Test Concentrations & Vehicles for 2800 Allergens*. Amsterdam, Elsevier Science Publishers B.V., 1986.
- Goon A T J, Bruze M, Zimerson E, Sörensen Ö, Goh C L, Koh D S Q, Isaksson M. Correlation between stated and measured concentrations of acrylate and methacrylate allergens in patch test preparations. *Dermatitis* 2011; **22**: 27–32.
- Goon A T J, Bruze M, Zimerson E, Sörensen Ö, Goh C L, Koh D S Q, Isaksson M. Variation in allergen content over time of acrylates/methacrylates in patch test preparations. *Br J Dermatol* 2011; **164**: 116–124.
- Mose K F, Andersen K E, Christensen L P. Stability of selected volatile contact allergens in different patch test chambers under different storage conditions. *Contact Dermatitis* 2012; **66**: 172–179.
- Frick-Engfeldt M, Zimerson E, Karlsson D, Skarpping G, Isaksson M, Bruze M. Is it possible to improve the patch-test diagnostics for isocyanates? A stability study of petrolatum preparations of diphenylmethane-4,4'-diisocyanate and polymeric diphenylmethane diisocyanate. *Contact Dermatitis* 2007; **56**: 27–34.
- Mowitz M, Zimerson E, Svedman C, Bruze M. Stability of fragrance test preparations applied in test chambers. *Contact Dermatitis* 2008; **58** (Suppl. 1): 31–41.
- Mowitz M, Zimerson E, Svedman C, Bruze M. Stability of fragrance patch test preparations applied in test chambers. *Br J Dermatol* 2012; **167**: 822–827.
- Isaksson M, Gruvberger B, Persson L, Bruze M. Stability of corticosteroid patch test preparations. *Contact Dermatitis* 2000; **42**: 144–148.
- Frick M, Zimerson E, Karlsson D, Marand Å, Skarpping G, Isaksson M, Bruze M. Poor correlation between stated and found concentrations of diphenylmethane-4,4'-diisocyanate (4,4'-MDI) in petrolatum patch test preparations. *Contact Dermatitis* 2004; **51**: 73–78.
- Ryberg K, Gruvberger B, Zimerson E, Isaksson M, Persson L, Sörensen Ö,

- Goossens A, Bruze M. Chemical investigations of disperse dyes in patch test preparations. *Contact Dermatitis* 2008; **58**: 199–209.
- 11 Nilsson U, Magnusson K, Karlberg O, Karlberg A T. Are contact allergens stable in patch test preparations? Investigation of the degradation of d-limonene hydroperoxides in petrolatum. *Contact Dermatitis* 1999; **40**: 127–132.
- 12 Fischer T, Maibach H I. Patch test allergens in petrolatum: a reappraisal. *Contact Dermatitis* 1984; **11**: 224–228.
- 13 Björkner B, Niklasson B. Influence of the vehicle on elicitation of contact allergic reactions to acrylic compounds in the guinea pig. *Contact Dermatitis* 1984; **11**: 268–278.
- 14 Bruze M, Björkner B, Lepoittevin J P. Occupational allergic contact dermatitis from ethyl cyanoacrylate. *Contact Dermatitis* 1995; **32**: 156–159.