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Evaluation of different injection techniques in the gas chromatographic determination of thermolabile trace impurities in a drug substance*

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Abstract: Thermodegradation of labile compounds in the hot injection port of a gas chromatograph causes many problems and can lead to ambiguous, poorly reproducible results. Splitless injection used in trace analysis in order to achieve a lower detection limit may even enhance the difficulties due to the longer residence time of the volatized sample in the injection port. Different techniques were tested to avoid thermodegradation of an epoxide and its corresponding chlorohydrin in a drug substance: variation of the injector temperature, high inlet flow rate during injection by electronic pressure programming, and cool on-column injection. The results showed that on-column injection was superior to splitless injection. The chlorohydrin formed epoxide by release of HCl upon splitless injection. A more detailed investigation of the factors that affect epoxide formation indicates that active sites within the injector are responsibile for the degradation and that the reaction is temperature dependent. However, results equivalent to those obtained by on-column injection were obtained for splitless injection when inert materials were used in the injector.

Keywords: *Gas chromatography; injection technique; thermolabile trace impurities.*

Introduction

The epoxide H 137/89 and its corresponding chlorohydrin H 240/18 are possible trace impurities in Almokalant (see Fig. 1 for structural formulae). The analytical method involves distribution between phosphoric acid and methylene chloride followed by gas chromatographic determination in the organic phase. Two gas chromatographic systems and different injection techniques were tested. Upon hot splitless injection the chlorohydrin released HCl and formed epoxide which led to poorly reproducible results. Losses of the epoxide were also observed. Splitless injection, with prolonged residence times of the sample in the injector means thermal stress to the analytes. A reduced residence time in the hot injector, achieved by a high initial flow rate, reduced degradation of the analytes, but it could not improve precision (Table 1). The reason was occasional leakage of the septum. On-column injection was superior to splitless injection both in terms of precision and of absolute peak areas. These results are summarized in Table 1. Details are published elsewhere [1].

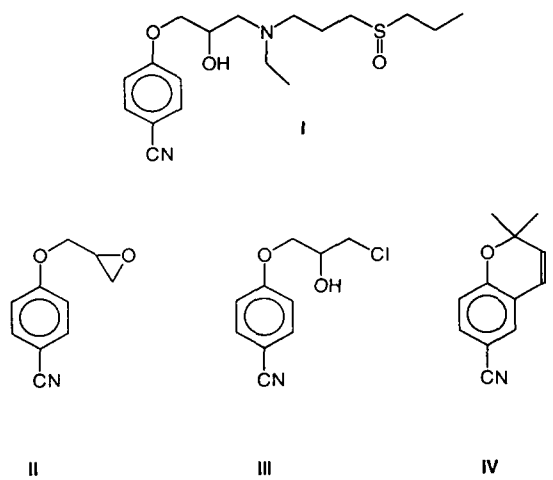

Figure 1
Structural formulae of Almokalant (I), H 137/89 (II), H 240/18 (III) and H 254/89 (IV, used as internal standard).

The present study focused on the degradation of the chlorohydrin during splitless injection. The purpose was to find out whether

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Table 1
Comparison of different methods and instrument models for the determination of the epoxide H 137/89 and the chlorohydrin H 240/18 in Almokalant

	Epoxide				Chlorohydrin			
GC model, method	RSD_{inj}† %	RSD_{met}‡ %	LOD§ $\mu g\ g^{-1}$	LOQ‖ $\mu g\ g^{-1}$	RSD_{inj} %	RSD_{met} %	LOD $\mu g\ g^{-1}$	LOQ $\mu g\ g^{-1}$
HP 5790 (splitless)	3.7 (n = 7)	9.2 (n = 5)	0.3	0.9	—	—	—	—
HP 5890 (splitless)	0.39 (n = 3)	2.6 (n = 5)	0.13	0.42	6.7 (n = 3)	3.1 (n = 5)	0.17	0.56
HP 5890 (EPP)*	0.32 (n = 3)	4.3 (n = 5)	0.22	0.72	3.7 (n = 3)	6.9 (n = 5)	0.35	1.2
HP 5890 (on-column)	0.19 (n = 3)	1.8 (n = 5)	0.09	0.29	0.64 (n = 3)	2.1 (n = 5)	0.09	0.31

*EPP: electronic pressure programming (high column head pressure during splitless injection).
†RSD_{inj}: relative standard deviation of the peak area ratio of the epoxide (chlorohydrin) and the internal standard for repeated injection of a standard solution (5 $\mu g\ ml^{-1}$).
‡RSD_{met}: relative standard deviation of the peak area ratios of epoxide (chlorohydrin) and internal standard in repeated samples of Almokalant spiked with 1 $\mu g\ g^{-1}$ epoxide or chlorohydrin, respectively.
§LOD: limit of detection.
‖LOQ: limit of quantitation according to [4].

splitless injection could be optimized so far that it could be applied instead of on-column injection for the determination of both epoxide and chlorohydrin. The peak shape of the chlorohydrin in splitless injection and the results of on-column injections indicate that degradation occurred in the injector. Chemical activity of injector glass liner and glass wool used as packing material, in combination with high temperatures, is usually blamed for creating such problems [2, 3]. Therefore different glass liners and packing materials were tested at different injector temperatures.

Experimental

The gas chromatographic procedure was as follows: instrument: Hewlett–Packard HP 5890 gas chromatograph equipped with a flame ionization detector, a capillary column split-splitless, and an on-column injector; column: 25 m × 0.32 mm i.d. fused silica capillary column coated with cross-linked methyl silicone (HP Ultra 1), 0.52-μm film thickness, coupled to a 5 m × 0.32 mm i.d. fused silica retention gap; injector temperature varied 180–230°C; detector temperature 290°C; oven initial temperature 40°C, 3 min; rate 30° min^{-1} to 200°C, then 8°C min^{-1} to 230°C, then 40°C min^{-1} to 270°C final temperature, 3 min; carrier gas helium, 0.69 bar initial column head pressure (1.8 ml min^{-1}); detector gases: hydrogen 35 ml min^{-1}, air 400 ml min^{-1}; make up (nitrogen) 35 ml min^{-1}.

The precision of the injection was tested by repeated injections of a standard solution containing 5 $\mu g\ ml^{-1}$ chlorohydrin and ca 4 $\mu g\ ml^{-1}$ H 254/89 (6-cyano-2,2-dimethyl-2H-1-benzopyrane) as internal standard. Dichloromethane was used as a solvent.

The following injector liners and materials were tested: (A) undeactivated glass liner, volume: 990 μl, with a plug of undeactivated glass wool; (B) undeactivated glass liner, volume: 990 μl, with a plug of hexamethyldichlorosilane (HMDS) deactivated glass wool; (C) same as B after some days of usage; (D) undeactivated glass liner, volume 990 μl, without glass wool; (E) deactivated glass liner, double-tapered, volume: 800 μl, without glass wool.

The injection volume was 1.7 or 2.0 μl depending on the volume of the glass liner. All injector modifications were tested at 180, 200 and 230°C injector temperature.

Results

The results are illustrated in Figs 2 and 3. The degradation of the chlorohydrin was strongly dependent on the materials in the injector. With an undeactivated borosilicate glass linear packed with a plug of raw, undeactivated glass wool (A) chlorohydrin could hardly be detected, and 77% was recovered as epoxide (200°C injector temperature). Using the same liner but HMDS deactivated glass wool instead (B), degradation could be

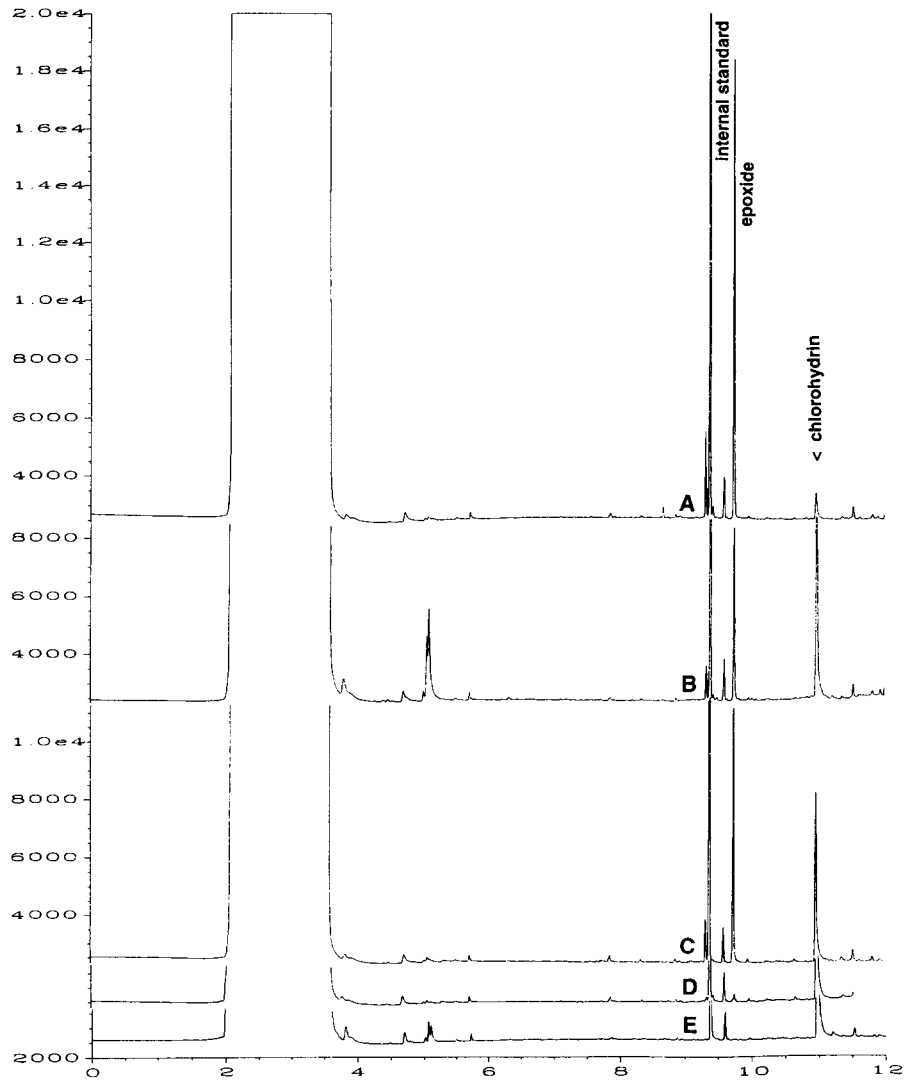

Figure 2
Chromatograms of a chlorohydrin standard solution (5 μg ml^{-1}) injected splitless at an injector temperature of 200°C, injector modifications A–E.

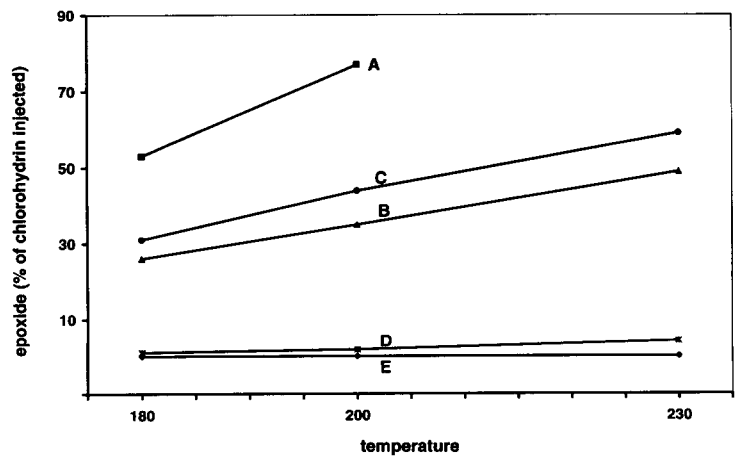

Figure 3
Temperature dependence of the formation of epoxide from chlorohydrin standard injected splitless using injector modifications A–E.

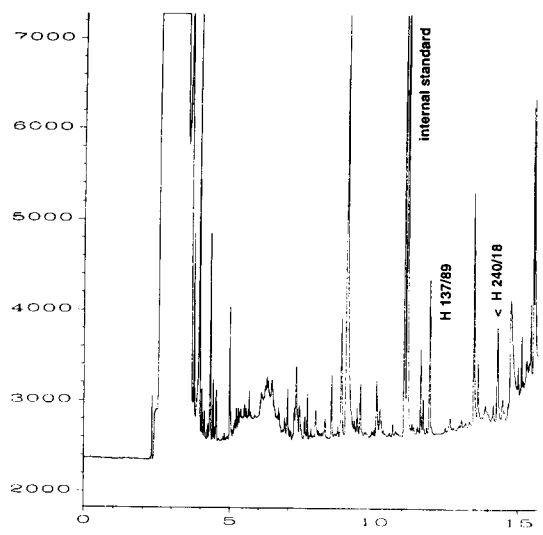

Figure 4
Chromatogram of a sample of Almokalant spiked with 1 μg g^{-1} of epoxide H 137/89 and chlorohydrin H 240/18 and injected on-column on the HP 5890 series II gas chromatograph.

reduced significantly and a reasonable chlorohydrin peak was obtained. However, after some days of usage the relative response of the chlorohydrin peak had decreased and the epoxide increased (C). This implies the formation of new active sites in the injector, probably due to reactivation of the glass wool surface. Both degradation of the chlorohydrin and epoxide formation were temperature dependent. When the same liner used in A–C was used without glass wool packing (D) chlorohydrin degradation and formation of epoxide could be reduced drastically, and with a deactivated double-taper borosilicate glass liner no epoxide was formed at any temperature tested.

Despite rather small amounts of analyte being injected into the chromatographic system the high recovery of the degradation product (epoxide) implies that adsorption and/or retention of the chlorohydrin in the injector is not a problem. The conversion of chlorohydrin into epoxide by the release of HCl seems to be catalysed by active sites introduced by the glass wool packing material in the injector liner. Deactivation of the packing material can, to some extent, prevent sample degradation. Similar results have been reported by Grob and Wagner [2] regarding split injections. The glass liner itself had only a minor effect on chlorohydrin degradation (comparing D and E).

For the chlorohydrin the precision of repeated injections at 200°C injector temperature was 0.69% RSD which is well comparable to the on-column injection. Applying different injector modifications, differences observed for repeated injections of an epoxide standard were not statistically significant.

Conclusions

In trace analysis of thermolabile trace components on-column injection is the first choice. A chromatogram of a spiked sample is shown in Fig. 4. However, the results show that splitless injection may produce equivalent results. This can be of interest in cases when on-column injection is not possible or not desired. The following points should then be considered:

- lowest possible injector temperature;
- deactivated, preferably tapered liners;
- avoid glass wool packing of the injector;
- high inlet pressure by EPP might improve results.

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