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Identification of caprolactam as a potential contaminant in parenteral solutions stored in overwrapped PVC bags

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Abstract: Semipreparative liquid chromatographic separation and subsequent off-line mass spectrometry have revealed caprolactam as a new contaminant in intravenous solutions. The content of the lactam was found to be 1.2–15.0 mg l^{-1} determined by liquid chromatography. Contamination is attributed to migration of caprolactam from the protecting plastic envelope through the PVC barrier and into the intravenous solution. Migration occurs during the final heat sterilization process.

Keywords: *Normal-phase LC; mass spectrometry; caprolactam; intravenous solutions; PVC bags.*

Introduction

Intravenous solutions stored in PVC bags are contaminated by small amounts of additives migrating from the plastic material. The plasticizer bis(2-ethylhexyl) phthalate (DEHP) has attracted extensive interest as a contaminant both of blood stored in PVC bags and of intravenous solutions; see for example refs 1 and 2. The contaminants 2-ethylhexyl hydrogen phthalate (mono ester), phthalic acid [3], phthalide [2] and epoxidized oils [4] have also been identified as migrants.

In preliminary work in which the migrants in old samples of intravenous solutions were monitored by LC, the major peak in the chromatogram was a contaminant not previously described.

The present paper describes the isolation of the unknown compound from an intravenous solution using semipreparative LC, followed by identification as hexahydro-1H-azepin-2-one (caprolactam) by mass spectrometry. The identity was confirmed by chemical reduction of an extract of the intravenous solution and subsequent gas chromatographic–mass spectrometric analysis of the resulting hexahydro-1H-azepine (hexamethylenimine). Finally, the content of caprolactam was determined using normal-phase LC and the source of the contamination was elucidated.

Experimental

Apparatus

Semipreparative separation was performed on a Waters System 600 liquid chromatograph fitted with a Wisp Model 712 auto-injector. The separated fractions were collected with a Gilson Model 201 fraction collector. Linking of the auto-injector and the fraction collector allowed repeated injections to be carried out automatically. For the semipreparative separations, a 250 × 8 mm i.d. column packed with 15–25 μm Lichroprep silica was used.

Quantitative determinations were performed on the same chromatograph using a 250 × 4.6 mm i.d. column packed with 5-μm Supelcosil/LC–SI. The detector wavelength was 210 nm for both separations.

The scanning of mass spectra was carried out by using an LKB 2091 gas chromatograph–mass spectrometer equipped with a direct inlet. The electron impact ion source was operated at 70 eV when scanning the mass spectra and at 14 eV for low energy spectra. The chromatography of the reduced caprolactam was performed on a fused silica capillary column (SPB-1, 30 m × 0.32 mm i.d., 0.25 μm film). The flow-rate of helium through the column was 1.4 ml min^{-1} and that of the make-up gas for the separator was 20 ml min^{-1}. The splitter was opened 30 s after injection of 2 μl into the

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column. The column oven was temperature programmed from 40 to 150°C at a rate of 4°C min^{-1}. The injector, separator and ion source were all maintained at 180°C.

Materials

The solvents used for the separations were of HPLC quality. Other reagents used were of analytical reagent grade.

Samples. Intravenous solutions dispensed in overwrapped PVC bags were commercial samples. Bags containing the following solutions were analysed: Sodium chloride 9 mg ml^{-1}, sterile water, Ringer acetate, Ringer lactate, glucose 50 mg ml^{-1}, glucose 50 mg ml^{-1} containing sodium chloride 9 mg ml^{-1}, mannitol 150 mg ml^{-1}, dextran 60 mg ml^{-1}, dextran 100 mg ml^{-1} and blood bags containing anticoagulant citrate phosphate dextrose and Sagman solution. The volume of the bags varied from 50 to 1000 ml (see Table 1).

Procedures

Semipreparative HPLC. Intravenous infusions (400 ml) with added sodium hydrogen carbonate (1.0 g), were extracted with chloroform (4 × 80 ml). After drying (over $MgSO_4$) and evaporation, the residue was dissolved in 2-propanol (1 ml) before LC. The mobile phase was *n*-hexane–2-propanol (3:1, v/v). The flow-rate was 2.5 ml min^{-1}, the injection volume was 150 µl and the retention time of caprolactam was 17–20 min.

Reduction of caprolactam. Lithium aluminium hydride (50 mg) was added to an extract of the intravenous solution (see above) dissolved in diethyl ether (10 ml). After reaction overnight, water (10 ml) was slowly added before extraction with ether (2 × 5 ml). The extract was dried (over $MgSO_4$) before analysis by gas chromatography–mass spectrometry. The retention time of the reduced compound was 7.00 min.

Extraction of plastic materials. To the plastic material (10 g) cut into small pieces was added 2-propanol (250 ml). After extraction for 10 days at room temperature, the solvent was decanted and evaporated. The residue was dissolved in *n*-hexane–2-propanol (1:1, v/v) to a total volume of 5 ml before liquid chromatographic analysis.

Quantitative determination of caprolactam. Octahydro-1H-azonin-2-one dissolved in methanol–water (1:1, v/v) was used as internal standard. For small bags (50–250 ml) an amount corresponding to 6 mg l^{-1} was used and for larger bags (500–1000 ml) an amount corresponding to 2 mg l^{-1} was used.

Sodium hydrogen carbonate (0.1 g) and a solution of internal standard (1 ml) were added to 50 ml of intravenous infusion. After mixing, the sample was extracted with chloroform (3 × 25 ml). After drying (over $MgSO_4$) and evaporation, the residue was dissolved in 2-propanol (5 ml) before LC analysis. The mobile phase was *n*-hexane–2-propanol (9:1, v/v). The flow-rate was 1.5 ml min^{-1}, the injection volume was 25 µl, the retention time of the internal standard was 14 min 30 s and the retention time of caprolactam was 17 min 33 s.

For each type of product (specifications

Table 1
Caprolactam contents of intravenous solutions in bags of different sizes

Solution	Caprolactam content (mg l^{-1})				
	50-ml Bags	100-ml Bags	250-ml Bags	500-ml Bags	1000-ml Bags
Sodium chloride (9 mg ml^{-1})	15.0	8.2	5.9	3.3	2.6
Sterile water	14.0	7.0			
Ringer acetate				2.4	2.8
Ringer lactate					1.8
Glucose (50 mg ml^{-1})		14.5	7.4	3.0	1.5
Glucose (50 mg ml^{-1}) + Sodium chloride (9 mg ml^{-1})				3.1	2.2
Mannitol (150 mg ml^{-1})				2.8	
Dextran (60 mg ml^{-1})				3.0	
Dextran (100 mg ml^{-1})				1.7	
CPD* solution (63 ml)				8.1	
Sagman solution† (100 ml)				5.9	

* Anticoagulant citrate phosphate dextrose.
† Anticoagulant and preservative. Not yet marketed.

according to British Pharmacopoeia 1988) a sample was prepared in glass equipment. Caprolactam was added to the preparation in the concentration range 0–30 mg l^{-1} and analysed as described above. Calibration graphs were constructed for each matrix. The linear responses of caprolactam and internal standard peak-height ratio were observed over the concentration range and the graphs passed through the origin of coordinates. The graphs were independent of the matrices. When blanks were analysed for contaminants, no trace of caprolactam was found.

Selectivity and precision. Migrants previously described in intravenous solutions stored in PVC bags did not interfere with the caprolactam determination.

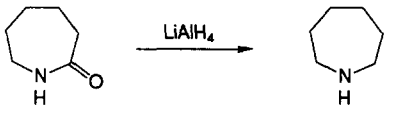
Ten samples of physiological saline solution prepared in glass equipment to which had been added 2.0 mg l^{-1} of caprolactam were analysed. A relative standard deviation of 0.68% was found.

Recovery and limit of detection. After extraction with 3 × 25 ml of chloroform, the recovery was calculated to be 93%.

The limit of detection by this method was about 0.02 mg l^{-1}; the signal-to-noise ratio was 3:1.

Results and Discussion

After semipreparative isolation of the unknown compound by LC, electron-impact mass spectra were recorded (70 and 14 eV) using the direct inlet. The spectra indicated a compound of low relative molecular mass (m/z = 113) with a base peak at m/z = 30. On the basis of its fragmentation pattern, the compound was suspected to be caprolactam [5]. The probability of the presence of the lactam was strengthened by comparing the fragmentation pattern and the retention time on the liquid chromatograph with that of an authentic sample. Since caprolactam was unexpected as a contaminant in the content of PVC bags, the extract was reduced with lithium aluminium hydride and analysed by gas chromatography-mass spectrometry. If the unknown was caprolactam (**I**), hexamethylenimine (**II**) should be formed. Both the fragmentation pattern and the retention time on the capillary column of the reduced sample corresponded to those of a reduced caprolactam standard and a commercial sample of hexamethylenimine.



I II

Figure 1
Chemical structures of caprolactam (**I**) and the product formed after lithium aluminium hydride reduction, hexamethylenimine (**II**).

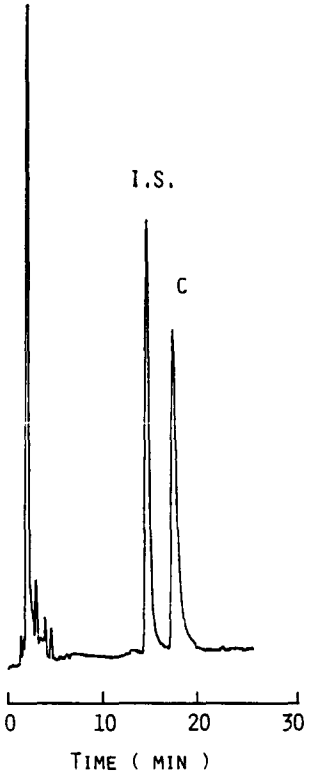


Figure 2
Representative chromatogram of caprolactam (C) and internal standard (i.s.) (octahydro-1H-azonin-2-one) from a chloroform extract of an intravenous solution containing sodium chloride 9 mg ml^{-1}. Column and conditions as in the text.

The contents of PVC bags from four manufacturers were analysed. The contaminant was detected in samples from three of the manufacturers. In bags from one manufacturer, caprolactam could not be detected. The positive findings are reported in Table 1. The smallest bags (50 ml) contained about 15 mg l^{-1} of caprolactam, the concentration decreasing with increasing size of the bag. The 1000-ml bag contained about 2 mg l^{-1} of caprolactam. Bags (500 ml, not shown in Table 1) manufactured in 1982/1983 showed a mean content of 6.9 mg l^{-1}, whereas the mean content of corresponding bags manufactured in 1987–1990 was 2.8 mg l^{-1}. Since no determination of

caprolactam was carried out when the former bags were manufactured in 1982/1983, it cannot be concluded that the content of caprolactam increases during storage. When six bags (500 ml) of sodium chloride 9 mg ml^{-1} solution from the same batch were analysed, the following contents of caprolactam were found: 3.2, 3.1, 3.2, 3.2, 3.1 and 3.2 mg l^{-1}. Uniformity of caprolactam content within the same batch was also found when analysing Ringer lactate solutions. Batch-to-batch variation for the same product obtained at the same time was also investigated. The difference between the maximal and minimal content of caprolactam in 100-ml bags containing sodium chloride solution was 0.8 mg l^{-1}; for the 500-ml bags the difference was 0.6 mg l^{-1}.

Reanalysis of new batches of dextran solutions from one of the three manufacturers showed no caprolactam at all. Investigation of the plastic materials confirmed that the manufacturer must have changed to new materials.

Analysis of the PVC material by HPLC revealed only traces of caprolactam (0.4 mg kg^{-1}). During the manufacturing process, the filled PVC bags are enclosed in a diffusion-tight envelope before the final heat-sterilization. Microscopy of the plastic material of the envelope revealed a laminated sheet. Three layers of polymer could be seen and between the polymer layers two layers of another material were found; this material was possibly a type of glue. Extraction and analysis of the plastic material of the envelope gave a much higher content of caprolactam (4.4 mg kg^{-1}) than that in the PVC material. The envelope could therefore be the source of the caprolactam.

In an unsterilized sample of 0.9% (w/v) sodium chloride solution kindly provided by one of the manufacturers, caprolactam was not detectable. A heat-sterilized bag from the same batch contained 1.2 mg l^{-1} of caprolactam. When the envelope was removed before the heat-treatment, caprolactam could not be detected.

Therefore, the caprolactam found must have migrated from the envelope through the PVC barrier into the solution during the heat treatment.

Caprolactam is classified as probably not carcinogenic to humans by the International Agency for Research on Cancer [6]. However, some positive findings are reported in a review of the genetic toxicity of caprolactam. The compound was considered to be a selective and weak genotoxin [7]. Further, caprolactam has given skin changes in the form of peeling and fissuring among workers who were exposed to atmospheric caprolactam [8]. The changes, however, were not associated with systemic toxicity. In white rats allergic reactions of caprolactam have been reported at a dose of 0.15 mg kg^{-1} administered orally. During study of the influence of autoimmune processes on developing rat foetuses, it was found that auto-antibodies in the blood serum produced significant embryotoxic effects [9].

Conclusions

Caprolactam has been identified as a new contaminant in intravenous solutions stored in overwrapped PVC bags. The protecting plastic envelope proved to be the source of the contaminant; its migration through the PVC barrier was prompted by the final heat treatment.

A LC method has been developed for the quantitative determination of caprolactam in commercial samples.

A consequence of the findings is that greater weight should be given to the selection of the plastic materials used in the protecting envelope.

Of wider implications, the potential migration of caprolactam ought to be investigated in other pharmaceutical products and cosmetics where polymers of caprolactam are used as packaging or as components of packaging materials.

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