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A new simple and rapid HPLC method for determination of DEHP in PVC packaging and releasing studies

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Abstract

A new simple, rapid and sensitive reverse-phase liquid chromatographic technique for the determination of di-2-ethylhexyl phthalate (DEHP), the major plasticizer of most PVC materials, is proposed. The proposed method is characterised by a single liquid/liquid hexane extraction procedure which can be used either for direct DEHP determination in PVC raw material or for the analysis of leached DEHP in the infusion bags during the perfusion process. The detection of DEHP is performed based on UV absorbance at 270 nm after direct injection of the hexane extract in a miscible eluent consisting of an acetonitrile/methanol mixture (9:1, v:v). The chromatographic process takes place on a classical C_{18} stationary phase. Linearity of the method is assumed from 0.25 to 5 ppm of DEHP with an acceptable repeatibility and reproducibility (RSD lower than 1.5 and 2.5%, respectively). Trace analysis is shown to be possible with respect to the limit of detection of 0.05 ppm. Moreover, the interesting possibility of using a reduced internal diameter column (2.1 mm) is underlined with respect to the resulting increase in sensitivity (0.01 ppm detectable). Some practical pharmaceutical applications are presented in order to demonstrate the reliability of the proposed method for the determination of DEHP in PVC packaging, as well as for traces of DEHP leached in infusion bags.

Keywords: PVC; Di(2-ethylhexyl) phthalate; Medical packaging; Leaching; HPLC

1. Introduction

Until the 1930s, the main commercial thermoplastic material was celluloid. During that decade polyvinyl chloride (PVC) plastics became commercially available and di(2-hexylphtalate) (DEHP) was first synthesized (Postaire, 1991). The flexibility brought to PVC by DEHP provided a significant advantage over celluloid and contributed to the rapid growth in its use. Since PVC is a hard, brittle and inflexible material, diethylhexylphtalate (DEHP) is predominantly added to impart flexibility. There are around 20 different phthalic anhydride esters (PAES) in current use, mostly as plasticizers, and they can be found in products representing construction materials, consumer goods, medical products and packaging. Among them, DEHP is characterised by its hydrophobicity and thus, its poor solubility in

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water. However, DEHP was suspected to be carcinogenic, hepatotoxic and teratogenic, and it has been shown to be released from PVC products containing lipophilic solutions (see, for example, Durmortier et al., 1990; Berg and Mayor, 1991; Dine et al., 1991; Elam and Nygren, 1992).

There are numerous methods for the determination of DEHP described in the literature essentially by gas chromatography (Venkataramanan et al., 1986; Hamon et al., 1987) HPLC (Haughey et al., 1988; Boselli and Cislaghi, 1991) and spectroscopy (European Pharmacopoeia, 1993) as official methods. They often differ in the extraction procedure involved. Nevertheless, these techniques and especially the extraction step are often tedious and time consuming (European Pharmacopoeia, 1993). This is the reason for which we wish to develop a fully automatizable, simple and rapid HPLC technique of extraction and detection of DEHP especially convenient for industrial quality control, either for raw material analysis or for trace analysis in infusions.

The reliability of the method was then evaluated in comparison to the European Pharmacopoeia method and the applicability to simulated infusions was determined.

2. Material and methods

2.1. Chemicals

All the solvents were of HPLC grade (Merck, Germany), and Milli-Q® (Millipore Corp., Bedford, MA) quality water was used. Special care should be taken with hexane, and its purity was carefully checked prior to analysis by HPLC under the conditions of use.

Di(2-ethylhexyl phtalate) (DEHP) was purchased from Merck, Darmstadt, Germany.

Two commercial brands of PVC medical packaging (100 ml PVC bag) were tested in this study.

2.2. Apparatus

Liquid chromatographic analysis was performed using a Gilson metering pump (model 401) (Villiers-le-Bel, France) equipped with a Gilson model 231 auto sampler which delivered 20 μl of the sample to be tested. Chromatographic separation was performed on ODS C_{18} (20 cm × 4.6 mm i.d.) and (20 cm × 2.1 mm i.d.) columns (Shandon, Cergy-Pontoise, France). The eluent was monitored at 270 nm with an SPD 6A UV detector (Shimadzu Corp., Kyoto, Japan). Separations were performed with a mobile phase consisting of an acetonitrile/methanol mixture (9:1 v/v), pumped at a flow rate of 0.8 and 0.17 ml/min for 4.6 and 2.1 mm columns, respectively.

2.3. Solutions

A 1% v/v stock solution of DEHP in hexane was kept at 4°C, protected from light for no more than 1 week.

Working (500 ppm) hexane solution was then prepared as needed.

Calibration was performed with diluted working solutions in hexane ranging from 0.25 to 5 ppm.

2.4. Sample preparation

2.4.1. Direct determination of DEHP in PVC raw material

Samples were prepared as described in the European Pharmacopoeia or as follows: 1 g of finely cut pieces of material was introduced into a glass stoppered tube (15 ml) and extracted at room temperature with 10 ml of hexane for 15 min (Agitelec, Paris, France).

After centrifugation (5 min at 5000 rpm), the supernatant was injected after appropriate dilution with the mobile phase in the chromatograph or analysed by UV spectroscopy according to the European Pharmacopoeia. All the assays were performed in triplicate.

2.4.2. Simulated perfusion and leaching studies

100 ml PVC bags were filled either with glucose 5% or NaCl 0.9%. Two cases were envisaged: liberation of DEHP after a contact period of 48 h at 25°C and after the simulation of perfusion (3 h at 25°C).

In both cases, 10 ml of the contents were extracted once with 10 ml of hexane under the

conditions described above. Then, direct injection of the solvent extract was performed. All the measurements were performed in triplicate.

3. Results and discussion

3.1. Analytical characteristics and validation of the proposed HPLC method

As an example, Fig. 1c displays a typical chromatogram obtained from an extract of a 0.9% NaCl solution contained in a commercial PVC bag. Due to the difficulty of obtaining a PVC material free of DEHP, the specificity of the determination was checked by the method of standard addition (Bolton, 1984; McCormick and Roach, 1987; Massart et al., 1988). The linearity obtained in such a way demonstrated the absence of interference.

For simplicity, the calibration was directly performed in hexane (Fig. 1b). It should be noted that the methanol/acetonitrile mixture (1:9 v/v) used as an eluent allowed the direct injection of the hexane extract, which greatly simplified the handling of the sample with respect to that described in the literature (European Pharmacopoeia, 1993). On the other hand, there is no evaporation step with the risk of loss of DEHP. Moreover, the absence of important disturbances of the background signal after injection should be stressed due to the total miscibility between the extract and the eluent.

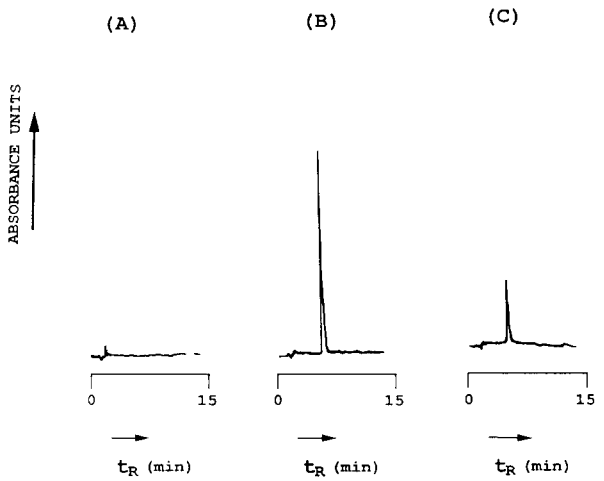

Fig. 1. Typical chromatogram of a DEHP extract by the proposed method (for chromatographic conditions see text). (a) Hexane blank. (b) DEHP calibration standard: 0.5 ppm. (c) Leaching study: (48 h, 25°C) chromatogram from an NaCl 0.9% solution contained in a 100 ml PVC bag as described in the text (estimated DEHP level: 0.19 ppm).

Table 1
Analytical characteristics of the proposed method

DEHP concentration (ppm)	Repeatability [a] (RSD %)	Reproducibility (RSD %)
0.25 ($n = 6$)	1.55	2.78
0.5 ($n = 6$)	1.06	1.10
1 ($n = 6$)	1.2	3.72
2.5 ($n = 6$)	0.10	1.20
5 ($n = 6$)	0.50	0.85

[a] $n = 10$ for each level of concentration.

Table 1 summarizes the main analytical characteristics of the proposed technique.

Linearity of the method is assumed from 0.25 to 5 ppm of DEHP ($r = 0.9999$), with a linearity test being highly significant ($p < 0.001$). Thus, the reported analytical characteristics and the limit of detection (LOD) of 0.05 ppm for a signal/background ratio of 3 as well as the limit of quantification (0.15 ppm) allow the use of the proposed technique either for the determination of DEHP in PVC raw material or for the detection of trace amounts in release studies.

Thus, the proposed method appeared well suited for automatic routine analysis due to a very simple sample pretreatment, the possibility of direct injection of the extracts and a reasonable time of analysis (< 10 min for each run). Moreover, the use of an internal standard was demonstrated as unnecessary as shown by the results of the validation study.

The yield of the liquid/liquid (L/L) extraction procedure was established. Table 2 reports the results obtained on six aqueous solutions spiked with different amounts of DEHP. The samples are then extracted as described in section 2. Complementary studies have shown that a second L/L extraction slightly improves the yield up to 95% but also the variability of the process (RSD > 3%), and on the other hand, increasing the

Table 2
Extraction yield of the liquid/liquid hexane procedure from water spiked with different amounts of DEHP

Spiked concentration of DEHP	Averaged recovery (ppm)	Yield (%)
5 ppm ($n = 5$)	4.65	93.0 ± 0.2
10 ppm ($n = 5$)	9.15	91 ± 0.15
20 ppm ($n = 5$)	18.22	91.1 ± 0.09

For experimental conditions see text.

involved volume of hexane does not lead to better results.

Therefore, a single L/L extraction was finally chosen for simplicity and rapidity.

3.2. Comparison of the proposed method with the European Pharmacopoeia method

In order to determine the DEHP content of the PVC raw material used in the pharmaceutical field, a comparison of the proposed HPLC method here described with the official European Pharmacopoeia method was undertaken. For this purpose, we attempted to evaluate firstly the extraction process itself, keeping the same method of analysis (i.e., HPLC) and secondly to compare the whole pharmacopoeial method with the proposed procedure (hexane L/L extraction and HPLC-UV detection). In such a way, the specificity of the direct UV detection described in the European Pharmacopoeia can be indirectly evaluated. Table 3 lists the results obtained according to the described protocol.

Comparison between the two first columns of Table 3 demonstrated (Student's t-test) the absence of significant difference (with $\alpha = 0.05$). This result, due to a common extraction step (EtOH/37°C), indirectly underlined the specificity of the direct UV detection of the official pharmacopoeial method.

On the other hand, one-way analysis of variance demonstrated that the proposed method (the third column) yielded significantly greater results ($p < 0.05$) in comparison with the other two methodologies. This fact suggested the higher efficiency of the L/L hexane extraction with regard to the use of alcohol heated at 37°C as used in the first two assays.

3.3. Use of the proposed technique for leaching studies

Release studies from plastic material are often mandatory to demonstrate the absence of interactions between the content of the medical packaging and the plastic material itself (Stiles et al., 1989; Cafmeyer and Wolfson, 1991; Krishnan et al., 1991; Waugh et al., 1991; Ulsaker and Teien, 1992; Airodo et al., 1993). In addition, as a large part of the infusion bags are in PVC, the DEHP determination in the infusions needed to be achieved with a sensitive and specific analytical method. Indeed, the generally large volume of the infusion (often over 100 ml) involving a considerable dilution of the leached DEHP, and the possible presence of a UV-absorbing drug added to the infusion can hamper the DEHP determination especially with direct UV detection. Due to

Table 3
Determination of the DEHP content of a PVC raw material (of quality complying with the European Pharmacopoeia)

	European Pharmacopoeial method	Extraction according the European Pharmacopoeia and analysis according to the proposed HPLC method	The proposed method (L/L extraction + HPLC detection)
Number of assays	$n = 6$	$n = 6$	$n = 6$
DEHP concentration (ppm)	806 ± 10.2	865 ± 40.3	1 001 ± 6.3

(a) Whole pharmacopoeial method; (b) extraction according to the pharmacopoeia combined with the use of HPLC as described here; (c) results from the whole proposed method.

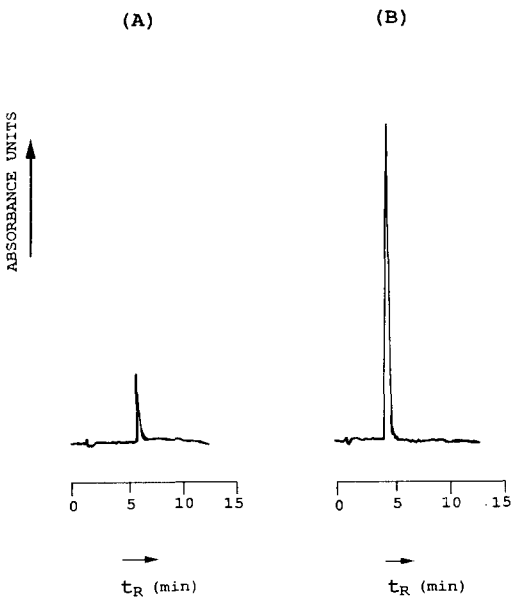

Fig. 2. Example of chromatogram corresponding to the same extract as in Fig. 1. (a) Classical column (i.d. = 4.6 mm) (b) Reduced diameter column (i.d. = 2.1 mm) (for chromatographic conditions see text).

the above-mentioned constraints, attempts to demonstrate the use, in terms of sensitivity and specifity, of the proposed technique were performed. Thus, a modified assay procedure was briefly evaluated in order to reach lower levels of released DEHP in infusions (isotonic sodium chloride injection in these experiments). For this purpose, the same analytical procedure as mentioned above was used. Nevertheless, a reduced diameter column (2.1 mm) filled with the same stationary phase was tested instead of the conventional diameter (4.6 mm, Fig. 2a). As an example, Fig. 2b illustrates the interest of a reduced column diameter with respect to the classical column (internal diameter, 4.6 mm). As shown, even without any adaptation of the unchanged injected volume (20 μl) and the flow cell (8 μl) of the detector, as theoretically needed by the reduction in column diameter (Novotny and Ishud, 1985; Rosset et al., 1991; Hagan and Weimann, 1992), a drastic increase in detectability of DEHP can be observed ($\times 4.6$) which is consistent with the expected increase predicted by the chromatographic theory, i.e., $(4.6/2.1)^2 \approx 4.7$.

This last point suggested that the contribution of the injected volume and the flow cell as well as the connection tubing to the broadening of the peak remained limited. With such an adaptation, the LOD was estimated to be 0.01 ppm; the linearity range comprises between 0.05 and 5 ppm ($r = 0.999$; with a linearity test F highly significant ($p < 0.001$)). The repeatibility ($n = 6$) remained very similar to that obtained with a conventional column (i.e., RSD = 1.7% for 1 ppm; 1.2% for 0.25 ppm and 2.61% for 0.05 ppm). Finally, intraday reproducibility ($n = 6$) seems convenient, i.e., 2.7% for 0.5 ppm and 3.9% for 0.05 ppm. Concerning the specificity, chromatograms issued from spiked isotonic glucose or sodium chloride infusions with some selected drugs absorbing in the UV range (i.e., bleomycin, fluroquinolones, etoposide, etc.) demonstrated the absence of interference whith the DEHP peak, certainly due the composition of the mobile phase.

Hence, using a reduced diameter column without any adapting device seems to be well suited for DEHP trace analysis in parenterals of large volume especially because of the improved limit of detection, and can be considered as an interesting alternative when very high sensitivity is needed.

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