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Method development and validation for the GC-FID assay of tributyl phosphate in a phospholipid emulsion

Eric Kesslen *, Ben Thomas

aai International, 6101 Quadrangle Drive, Chapel Hill, NC 27514, USA

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Abstract

This paper describes the development and validation of an isothermal GC-FID method for the assay of tributyl phosphate in a phospholipid emulsion. The emulsion is used as a topical ointment to deliver Triton X-100, a spermicide. The tributyl phosphate is added to the emulsion as a plasticizer or softening agent. The chromatographic conditions of the method employ a J&W DB-Wax capillary column (30 m × 0.53 mm, film thickness 1 μm), isothermal elution with He at a column flow of 2.0 ml/min, injector, detector, and oven temperatures at 210 °C, a split ratio of 18.0/2.0, and a 3-μl injection volume. Sample calibration was performed with tributyl phosphate purchased from Aldrich (USP Reference Standard is not available). The linearity of the tributyl phosphate peak area responses was demonstrated from approximately 50 to 150% of the analytical concentration of 100 μg/ml. System precision was determined from five replicate injections of a standard and sample solution. Reproducibility of the tributyl phosphate peak area responses showed R.S.D. of 1.2 and 0.4%, respectively. Method precision was performed by assaying five samples by two different analysts on different days. The mean %LC was 95.5% (R.S.D. = 1.0%) for the first analyst, and 95.6% (R.S.D. = 1.0%) for the second analyst. The mean %LC value for all ten sample preparations was 95.5% (R.S.D. = 0.9%). The limits of detection and quantitation were determined to be 0.2 and 0.7 μg/ml, respectively.

Keywords: Tributyl phosphate; Gas chromatography; Assay; Development; Validation

1. Introduction

The method development for the assay of tributyl phosphate was based on its chemical properties. The USP does not have an assay method for tributyl phosphate and quantitative assays found in the literature are not appropriate for high sample throughput or pharmaceutical samples [1–4].

Tributyl phosphate, $(CH_3CH_2CH_2CH_2–O)_3–P=O$, is a polar molecule and, therefore, a moderately polar solvent, CH_2Cl_2, was used as the diluent and a polar column, DB-Wax, was used for separation. The GC parameters used in the method development were based on the boiling point (289 °C) and the flash point (144 °C) of tributyl phosphate. The injection port, detector and oven temperatures were all set to 210 °C. The

* Corresponding author. Tel.: +1-919-493-5718; fax: +1-919-493-9404.

E-mail address: eric.kesslen@aaiintl.com (E. Kesslen).

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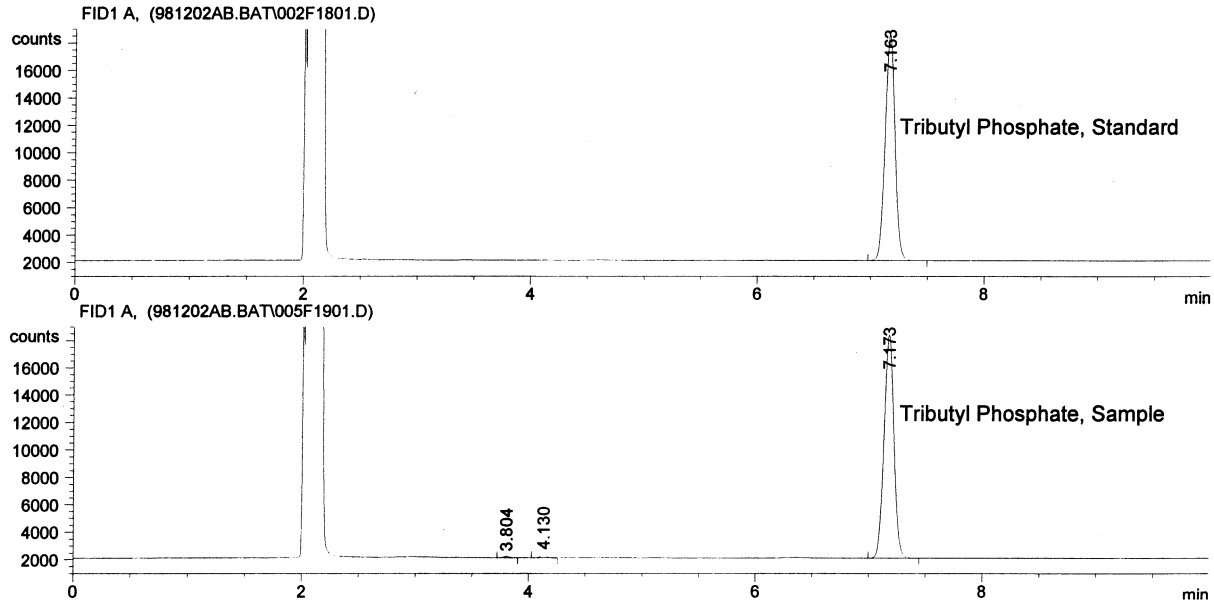

Fig. 1. Sample and standard chromatograms.

oven program is isothermal with a run time of 10 min. The head pressure was set to ensure a He flow of 2.0 ml/min. The split was then adjusted to 18.0/2.0. The solvent, column and acquisition parameters were chosen to be a starting point for the method development. However, the chromatography produced using these parameters was excellent. The retention time of tributyl phosphate was approximately 7 min with good peak shape and USP tailing was approximately 1.0. No further development was required.

Additionally, preliminary precision and linearity studies performed during the development of the method showed that the 3 μl injection volume was reproducible and the peak response was significant at the analytical concentration. This precluded the need for an internal standard.

A serial extraction of the emulsion in water using CH_2Cl_2 proved inefficient and recoveries were less than 10%. Diluting the emulsion in CH_2Cl_2 gave solutions that could be injected directly (without dilution, filtration or centrifugation). Chromatograms of the resulting solutions gave very good peak shapes (USP tailing ca. 1.0) and co-elution of excipients was not observed.

Table 1
System precision

System precision		
Sample	Response (counts/s)	Retention time (min)
Injection 1	98 569.8	7.172
Injection 2	97 531.9	7.174
Injection 3	98 229.9	7.173
Injection 4	98 378.4	7.175
Injection 5	97 952.2	7.177
Mean (5)	98 132.4	7.1742
%R.S.D.	0.4	<0.1 (0.03)
Standard		
Injection 1	99 435.9	7.164
Injection 2	99 948.2	7.165
Injection 3	101 292	7.165
Injection 4	99 647.5	7.168
Injection 5	10 2316	7.166
Mean (5)	100 528	7.1656
%R.S.D.	1.2	<0.1 (0.02)

Table 2
Method precision/intermediate precision

Method precision/intermediate precision		
Sample	%Label claim	
	Analyst 1	Analyst 2
Preparation 1	94.8	
Preparation 2	96.7	
Preparation 3	94.3	
Preparation 4	96.2	
Preparation 5	95.3	
Preparation 6		94.4
Preparation 7		95.7
Preparation 8		96.1
Preparation 9		96.8
Preparation 10		95.1
Mean (5)	95.5	95.6
%R.S.D.	1.0	1.0
Mean (10)	95.5	
%R.S.D.	0.9	

Table 3
Linearity parameters for tributyl phosphate

Calibration range	50–150 Nominal analytical concentration (%)
Concentration (μg/ml)	50–150
Y intercept	−5337.3
Slope	998.9
Correlation coefficient, r	0.99993
%Y intercept	−5.7

2. Experimental

2.1. Materials

The methylene chloride used was purchased from Fisher Scientific and purity was 99.9%. Tributyl phosphate reference material was purchased from Aldrich and purity was 99.1%.

2.2. Equipment

A Hewlett-Packard model 5890 gas chromatograph equipped with a flame ionization detector was used to chromatograph the solutions. Separation was achieved using a J&W DB-Wax capillary column with the following dimensions, 30 m × 0.53 mm and 1 μm film thickness. The data was acquired via HP CHEMSTATION Data Acquisition Software, Version A.04.01. A Mettler AG245 analytical balance was used for massing standards and samples and a Branson 8210 Sonicator was used for sample dissolution. The chromatographic conditions are listed below.

Carrier gas, He.
Detection, FID.
Injector, detector and oven temperatures, 210 °C.
Rate is 0 °C/min (isothermal).
Flow rate, 2.0 ml/min.
Split ratio, 18.0/2.0.
Injection volume, 3 μl.
Quantitation, peak area.
Approximate retention time, tributyl phosphate, 7 min.
Run time, 10 min.

3. Preparation of solutions

3.1. Standard preparation

A stock solution at 1 mg/ml was prepared by dissolving 100 mg of tributyl phosphate in 100 ml of methylene chloride. A 10 ml aliquot of the stock solution was diluted to 100 ml in methylene chloride, yielding a final concentration of 0.1 mg/ml.

3.2. Sample preparation

The phospholipid emulsion is 1% (w/w) in tributyl phosphate. A 0.1 mg/ml solution was prepared by dissolution of 1 g of the emulsion in 80 ml of methylene chloride with sonication. The final volume was adjusted to 100 ml with methylene chloride.

See Fig. 1 for examples of standard and sample chromatograms.

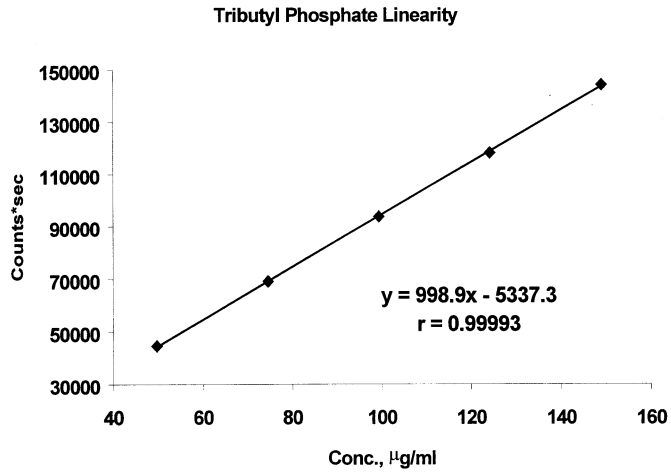

Fig. 2. Range of linearity.

4. Results

4.1. Precision

4.1.1. System precision

The precision of the tributyl phosphate peak area response and retention time was assessed from five replicate injections of standard and sample solutions. The data is summarized in Table 1.

4.1.2. Method/intermediate precision

The precision of the method for the emulsion was assessed by the assay of five samples containing the nominal amount of tributyl phosphate. Intermediate precision was studied by assaying five samples prepared by a different analyst, using a different GC column, on a different day. The results are reported in Table 2.

4.2. Range of linearity

The linearity of peak area response versus concentration for tributyl phosphate was studied from approximately 50 to 150 μg/ml. Five solutions were prepared corresponding to 50, 75, 100, 125 and 150% of the nominal analytical concentration (100 μg/ml). The method was found to be suitable for a single point calibration. The data is summarized in Table 3 and plotted in Fig. 2.

4.3. Accuracy/recovery

The recovery of tributyl phosphate from the phospholipid emulsion was studied by assaying placebo samples spiked with the active, corresponding to a final concentration of 50, 100, and 150% of the nominal analytical concentration of 0.1 mg/ml. The results are summarized in Table 4.

Table 4
Accuracy/recovery for tributyl phosphate from the phospholipid emulsion

Accuracy/recovery			
Level	%Recovery	Mean (4)	%R.S.D.
50%	102.0	101.6	0.4
50%	101.9		
50%	101.5		
50%	101.0		
100%	100.8	100.8	0.6
100%	100.1		
100%	101.5		
100%	100.8		
150%	100.3	100.2	0.2
150%	100.0		
150%	100.0		
150%	100.3		
Mean (12)	100.9		
%R.S.D.	0.7		

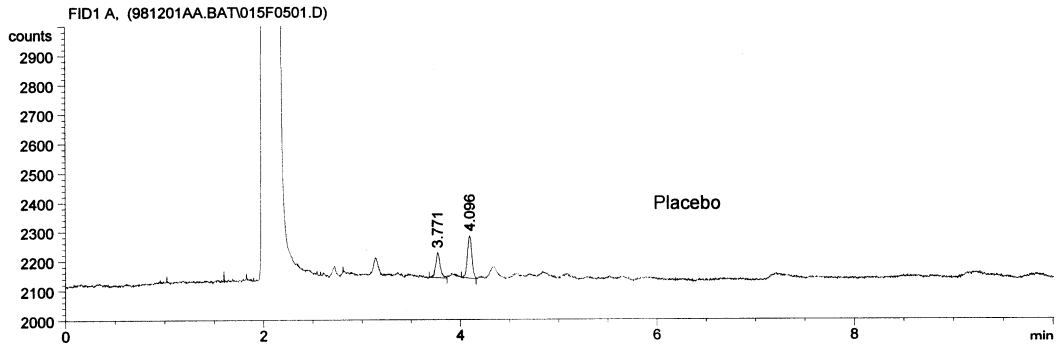

Fig. 3. Chromatogram of placebo.

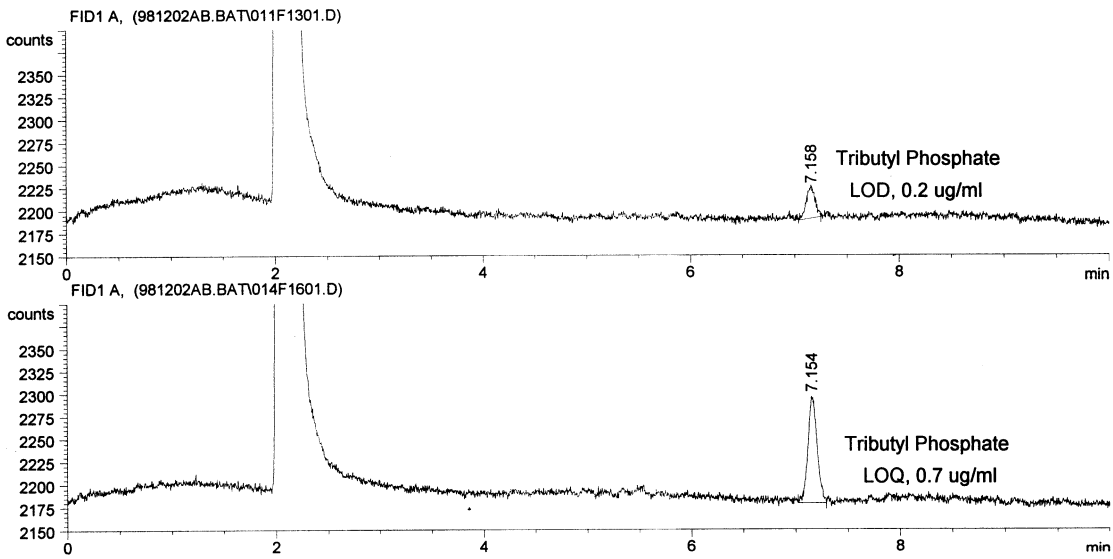

Fig. 4. Chromatograms of LOD and LOQ.

4.4. Selectivity

Injections of the extracted placebo were performed to demonstrate the absence of interference with the elution of tributyl phosphate. No interference was observed, (Fig. 3).

4.5. Sensitivity

The limit of detection was determined to be 0.2 μg/ml with a S/N ratio of 2.7. The limit of quantitation was 0.7 μg/ml with a S/N ratio of 8.5. The relative standard deviation for three injections of the LOQ solution was 4.2%. This measure of reproducibility ensures that tributyl phosphate at the LOQ level can be integrated reproducibly. Fig. 4 shows chromatograms of tributyl phosphate at LOD and LOQ levels.

5. Conclusion

This method for assaying tributyl phosphate extracted from a phospholipid emulsion is capable of high sample throughput and applicable to pharmaceutical samples. Validation testing shows that the method is specific and linear from 50 to 150 μg/ml. The $\%Y$ intercept achieved during the

linearity testing is high at 5.7%, absolute. The $\%Y$ intercept is a calculated measurement of the deviation from the origin that a blank solution would have. The result for this parameter suggests that the method may have a bias. Specificity testing performed as part of this validation show no such bias, i.e. no coeluting peaks. Furthermore, the accuracy testing performed as part of this validation does not support any bias associated with this method. It is possible that the method is not linear at concentrations outside the range tested for this validation. The intent of the method was to be linear from 50 to 150% of the analytical concentration.

Precision studies show a high degree of reproducibility in both system precision and ruggedness testing. However, the results achieved during the method precision studies are low, 95.5% recovery for $n = 10$. Since the accuracy studies have a mean recovery of 100.9% (for $n = 12$ at three concentration levels) the low recovery observed during the method precision studies is attributed to the samples provided for the validation of this method.

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