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DETERMINATION OF GALLAMINE AND ITS IMPURITIES BY REVERSED-PHASE ION-PAIR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND COMPARISON WITH THIN-LAYER CHROMATOGRAPHY

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SUMMARY

A reversed-phase, ion-pair high-performance liquid chromatographic (HPLC) method for the determination of gallamine and its impurities is described. The separation is achieved on a Nucleosil C_{18} column with acetonitrile–aqueous phosphate buffer (pH 3.0) (31:69, v/v) containing 0.1 *M* sodium perchlorate as eluent and on-line UV detection at 200 nm. The method is sensitive (the detection limit is 0.7 ng injected) and reproducible, with a peak area coefficient of variation of 0.19% ($n = 15$; 3 μg injected) and 1.65% ($n = 15$; 10 ng injected) for a gallamine assay; the detector response is linear over the concentration range 0.5–250 μg/ml of gallamine triethiodide with a correlation coefficient of 0.9997. The method has been used to isolate the two main impurities contained in gallamine triethiodide batches; their structures have been determined by NMR and fast atom bombardment mass spectrometry. Various gallamine triethiodide batches have been analysed and the HPLC results compared with those obtained by thin-layer chromatography.

INTRODUCTION

Gallamine triethiodide (Fig. 1a), a substitute for curare, was first synthesized in France by Rhône-Poulenc in 1947. Since then, it has been used mainly as a non-depolarizing neuromuscular blocking agent in anaesthetic practice.

Several methods for gallamine triethiodide assay have been proposed. Some workers used dye-binding fluorescence methods[1–4] but these were not suitable for the assay of both gallamine and its impurities. Recently, two chromatographic methods[5,6] have been proposed for these determinations but the results were not very convincing.

This paper describes a method for the determination of gallamine triethiodide and its main impurities by high-performance liquid chromatography (HPLC). Two impurities were isolated by preparative chromatography and their structures were determined. The results obtained by HPLC and thin-layer chromatography (TLC)[7] were compared.

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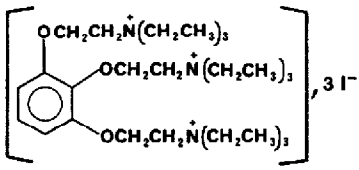

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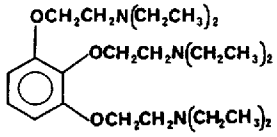

Fig. 1. Structures of (a) gallamine triethiodide and (b) gallamine base.

EXPERIMENTAL

Chemicals

Gallamine triethiodide samples were from different sources. HPLC-grade acetonitrile was purchased from Prolabo (Paris, France). Deionized water was further purified on a Milli-Q system (Millipore, Bedford, MA, U.S.A.). All other chemicals were of analytical-reagent grade from Prolabo.

Apparatus

The HPLC system consisted of a Gilson (Villiers-le-Bel, France) gradient chromatograph equipped with a Rheodyne 20-μl sample loop injector, a Gilson Model 231 autosampler, a Kratos (Ramsey, NJ, U.S.A.) Spectroflow 757 UV detector and a Perkin-Elmer (Wilton, CT, U.S.A.) LCI100 integrator recorder coupled with a Perkin-Elmer Model 7700 datastation. Detection was also carried out with a Hewlett-Packard (Palo Alto, CA, U.S.A.) Model 1040 A diode array detector.

The impurities were isolated with a semi-preparative chromatograph consisting of a Gilson Model 303 HPLC pump coupled with a Model 803C manometric module. Rheodyne 6-ml sample loop injector was used. The UV detector was an LDC Spectromonitor III (Laboratory Data Control, Riviera Beach, FL, U.S.A.) equipped with a 35-μl semi-preparative cell.

HPLC conditions

Chromatographic columns (25 cm × 0.46 cm I.D.) were packed with either 5-μm Nucleosil octadecyl-bonded silica, 5-μm Nucleosil cyanopropylsilica or 5-μm Nucleosil bare silica (mean pore diameters 100 Å) (SFCC, Gagny, France). Sodium perchlorate was added to the buffer–acetonitrile mobile phase. The aqueous part of the mobile phase was adjusted to pH 3 by adding sodium hydroxide to 0.05 *M* phosphoric acid solution. Mobile phases containing 0–0.5 *M* sodium perchlorate were prepared.

On the octadecyl bonded silica, either isocratic or gradient elution was used. For isocratic elution the mobile phase consisted of acetonitrile–20.3 g/l sodium perchlorate solution buffered at pH 3 (31:69, v/v). This mobile phase also gave good results on cyanopropyl-bonded silica. In some instances, gradient elution was neces-

sary. Two solvent mixtures were prepared: solvent A [acetonitrile–15.5 g/l sodium perchlorate solution buffered at pH 3 (10:90, v/v)] and solvent B [acetonitrile–40 g/l sodium perchlorate solution buffered at pH 3 (65:35, v/v)]. At time zero, the mobile phase was a mixture of 25% B in A, increased linearly to 50% in 20 min and to 65% after 25 min. This level was maintained until the end of the elution.

On bare silica, the best mobile phase was acetonitrile–17.5 g/l sodium perchlorate solution buffered at pH 3 (20:80, v/v). However, the octadecyl-bonded silica gave the best results.

Volumes of 20 μl of gallamine solutions were injected. The solutions were prepared by adding 0.05–40 mg of a gallamine triethiodide sample to 100 ml of mobile phase. The flow-rate was 1 ml/min. The analysis was performed at room temperature and the UV wavelength was 200 nm.

Preparative liquid chromatographic conditions

We used a 25 cm × 2.1 cm I.D. column packed with 5-μm Nucleosil octadecyl-bonded silica. The mobile phase composition was as specified above for isocratic elution and the flow-rate was 12 ml/min. A 100-mg amount of a gallamine triethiodide batch, added to 100 mg of sodium perchlorate and dissolved in 6 ml of mobile phase, was injected (a counter ion has to be added to prevent peak broadening). The UV wavelength was set at 230 nm instead of 200 nm to avoid UV saturation.

After collection, the fractions containing the impurities were immediately neutralized to avoid acidic degradation. Acetonitrile and part of the water were evaporated under vacuum at 30°C. The concentrated impurities were nearly insoluble in the aqueous phase overloaded with phosphate and perchlorate. Hence they were excluded from the aqueous phase and it was possible to collect them in the supernatant without contamination by sodium perchlorate of sodium dihydrogenphosphate.

TLC conditions[7]

Silica gel pre-coated flexible TLC sheets (20 cm × 20 cm) were purchased from Prolabo (F 1500, Schleicher et Schüll). The plates were activated at 110°C for 30 min and 5 μl of a 20 mg/ml methanolic solution of gallamine triethiodide were applied.

Development was achieved with acetone–11 *M* hydrochloric acid–water (50:25:25, v/v). After migration, the plates were dried and sprayed with a solution of 1 g of hexachloroplatinic acid dissolved in 4 ml of hydriodic acid (sp. gr. 1.7).

Structure determination

The mass spectra of the two unknown impurities were recorded by fast atom bombardment mass spectrometry (FAB-MS) using a Kratos AEI MS 50 mass spectrometer.

^{1}H NMR spectra were recorded at 250 MHz on a Bruker (Wissembourg, France) WM 250 instrument in DMSO-d_6 solution, using dimethyl sulphoxide (DMSO) as a reference (δ DMSO = 2.5 ppm). Coupling constants (J) were measured in hertz.

^{13}C NMR spectra were recorded at 50.3 MHz on a Bruker WP 200 SY instrument in DMSO-d_6 solutions, using DMSO as a reference (δ DMSO = 39.5 ppm). The carbon multiplicity was determined by a spin–echo J modulation experiment.

RESULTS AND DISCUSSION

Separation principles

Many drug substances contain nitrogen with basic properties. As these substances are often soluble in water and acetonitrile, reversed-phase HPLC seems to be a suitable technique for their determination. In water–acetonitrile mixtures, they cannot be chromatographed in their non-ionized from because the mobile phase is alkaline and damages the silica-based stationary phase. Consequently, a counter ion has to be added to an acidic mobile phase to allow ion pairing with the solute[8,9]. Often, however, tailing effects or even irreversible adsorption on the stationary phase are observed, probably owing to the presence of residual silanol groups in the stationary phase[10–13]. End-capped stationary phases[14,15] can reduce this tailing effect. Another remedy is to add anti-tailing agents such as long-chain quaternary ammonium[10,16–20] to the eluent. Even though the change may be impressive, the resulting mobile phase is complex; further, these agents are usually paired to anions (*e.g.*, bromide) absorbing at 220 nm, which is a major drawback for the detection of many compounds.

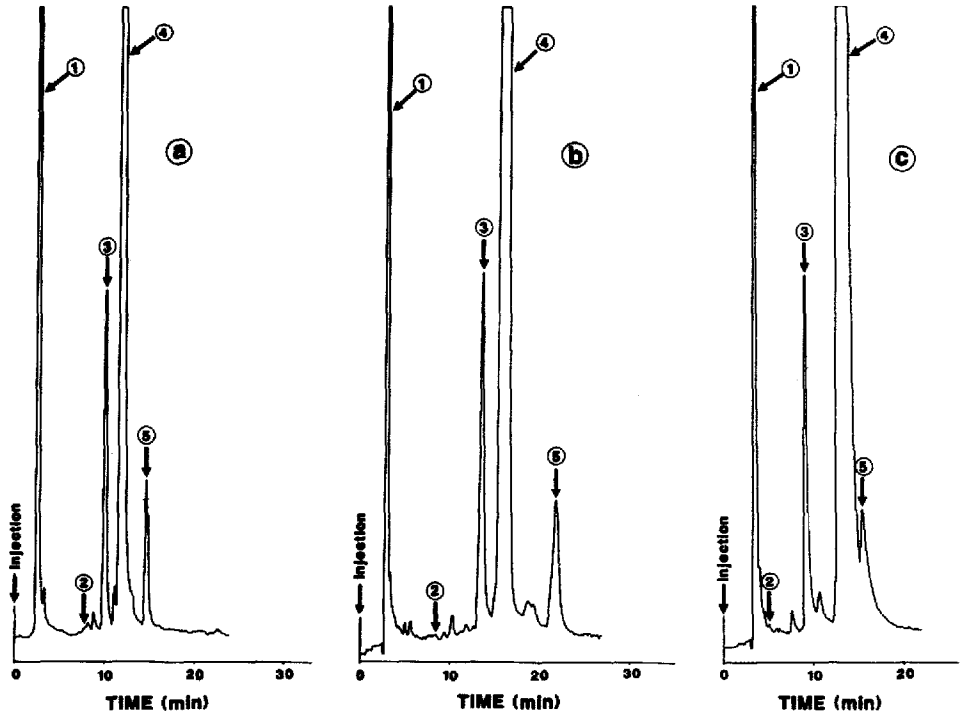

Fig. 2. Influence of the nature of the support on the separation of the main components of a gallamine triethiodide sample. Columns, 25 × 0.46 cm I.D.; stationary phase, (a) 5-μm Nucleosil octadecyl-bonded silica, (b) 5-μm Nucleosil cyanopropyl-bonded silica and (c) 5-μm Nucleosil silica. HPLC conditions: all mobile phases contained 0.1 *M* sodium perchlorate (pH 3); (a) and (b) acetonitrile–water (69:31, v/v); (c) acetonitrile–water (80:20, v/v). Flow-rate, 1 ml/min; detection, UV at 200 nm. Solutes: peaks 1 and 4, gallamine triethiodide; 2 represents the retention time of gallamine base; 3 and 5 are impurities. The appearance of two peaks for gallamine triethiodide is explained in the text.

On the other hand, a small lipophilic counter ion, such as perchlorate, can be used with the highly positively charged ammonium salts such as gallamine triethiodide in order to avoid peak broadening due to slow complex formation kinetics. There are many examples in the literature[13,21–23] in which perchlorates were used as counter ions to quaternary ammonium compounds without any peak tailing.

Retention mechanisms

Using acetonitrile as organic modifier and a perchlorate concentration of 0.1 *M*, the nature of the stationary phase has a minor effect on the retention of gallamine. The elution order is the same on octadecyl-bonded silica, cyanopropyl-bonded silica and bare silica (Fig. 2). Moreover, with these three supports, a decrease in the acetonitrile content of the mobile phase (at a constant perchlorate concentration) increases solute retention. The same phenomenon was observed by Abidi[21] on octadecyl-, cyanopropyl- and phenylpropyl-bonded silica with similar mobile phases.

We studied the dependence of retention on mobile phase perchlorate concentration (Fig. 3). The most important differences were observed between the silica and the two other bonded phases; they were significant at low sodium perchlorate concentration (below 0.1 *M*). Variations of the capacity factors with salt concentration were less pronounced over the concentration range 0.1–0.5 *M*.

The influence of sodium perchlorate on the retention on the cyanopropyl–bonded phase is similar to that on the octadecyl-bonded phase. The affinity of acetonitrile for the cyanopropyl bonds may be greater than that of water because of their chemical similarity. Consequently, the bonded groups could be surrounded by acetonitrile in the same way as octadecyl bonds, which could explain the similarities in chromatographic behaviour (Fig. 3a and b). The shape of the curve of k' *versus* sodium perchlorate concentration obtained is well known and has been thoroughly studied by many workers[24–26].

In reversed-phase ion-pair chromatography, two retention mechanisms are distinguished: the first stipulates the formation of ion pairs in the mobile phase prior to retention on the bonded support; the second predicts ion exchange between the cationic solute and the counter ion already adsorbed on the stationary phase[24–28].

Even though both mechanisms may occur together, the second probably predominates[25,26,28,29], at least with alkylsulphate or alkylsulphonate counter ions. With perchlorate, a much more hydrophilic counter ion, the first mechanism cannot be eliminated. If this mechanism were to predominate, however, the three different stationary phases, assumed to be free of adsorbed counter ions, would show great differences in selectivity and the mobile phase polarity would have opposite effects on retention on these supports. Consequently, the second mechanism based on ion exchange must also play a role. At high concentration, perchlorates are likely to cover the stationary phase pores entirely, whatever the support may be, and there would be ionic interactions between perchlorates and the quaternary ammonium of gallamine.

A decrease in solute retention on silica on increasing the counter ion concentration such as shown in Fig. 3c has already been observed[23,30–32]. Moreover, we observe that the influences of mobile phase polarity and of counter ion concentration were similar to those noted in a previous study[30–32]. Perchlorates are partly adsorbed on silica. The retention mechanism is probably based on ion exchange either directly on silanol (SiO^-H^+) groups (at low sodium perchlorate concentration) or on per-

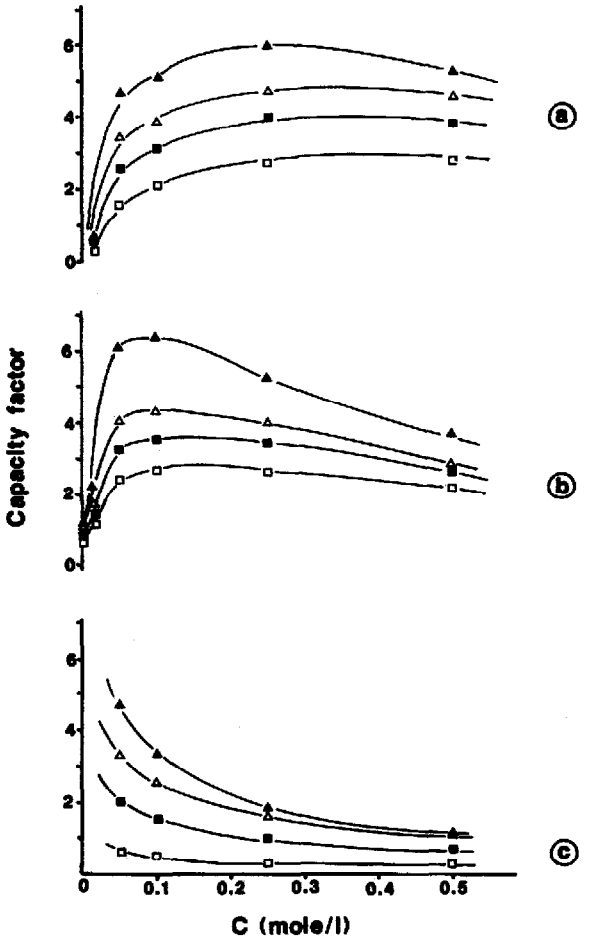

Fig. 3. Influence of the sodium perchlorate concentration in the mobile phase on the capacity factors of the gallamine derivatives. (a) 5-μm Nucleosil octadecyl-bonded silica; (b) 5-μm Nucleosil cyanopropyl-bonded silica; (c) 5-μm Nucleosil silica. HPLC conditions: aqueous phases buffered at pH 3; (a) and (b) acetonitrile–water (69:31, v/v); (c) acetonitrile–water (80:20, v/v). (□) Gallamine base; (■) impurity 3; (△) gallamine triethiodide (peak 4); (▲) impurity 5.

chlorate. The solute retention decrease with increase in sodium perchlorate concentration could be due to the substitution of perchlorate for silanols as interacting points and to the increase in solvent strength. The retention increase with decrease in acetonitrile content in the mobile phase is a consequence of the decreased perchlorate concentration in the stationary phase.

Nucleosil octadecyl-bonded silica was used in the remainder of this study; cyanopropyl-bonded silica also gave good separations but the results were less reproducible.

Identification of chromatographic peaks and optimization of detection parameters

Peak 1 identification was achieved using iodide; the chromatographic peaks ob-

tained after injection of sodium or potassium iodide solution (Fig. 2a) have the same retention times as peak 1. Moreover, the UV spectra [measured by diode array detection (Fig. 4a)] were similar. Hence we conclude that there is an immediate substitution of perchlorate for iodide on gallamine after injection. The iodides, paired with sodium ions, elute in peak 1. Gallamine base is at pH 3.0 triprotonated and coupled to three perchlorate ions; it is eluted in peak 2. Peak 4 detected in gallamine triethiodide batches is due to gallamine triethylperchlorate. We observe that the injected gallamine triethiodide batch contains two impurities (called impurities 3 and 5) corresponding to peaks 3 and 5.

Their structures, determined by NMR and mass spectrometry, are presented in Fig. 5. After their isolation by preparative chromatography, the impurities were paired to perchlorate, which confirms the ion exchange between iodide and perchlorate. Thus, in the gallamine batches, impurities 3 and 5 are gallamine diethiodide and 1,2,3-tris(2-triethylammonioethoxy)-4- (2-triethylammonioethyl)benzene tetraiodide, respectively.

Optimization of the detection wavelength was achieved with a diode array detector. Maximum absorbance of gallamine triethiodide is at 225 nm (Fig. 4b). Gallamine triethiodide, however, contains two chromophores: the aromatic ring and the iodide ions (Fig. 4a). Maximum UV absorbance at 225 nm is due to iodide instead of gallamine. The gallamine UV spectrum, obtained by diode array detection on peak

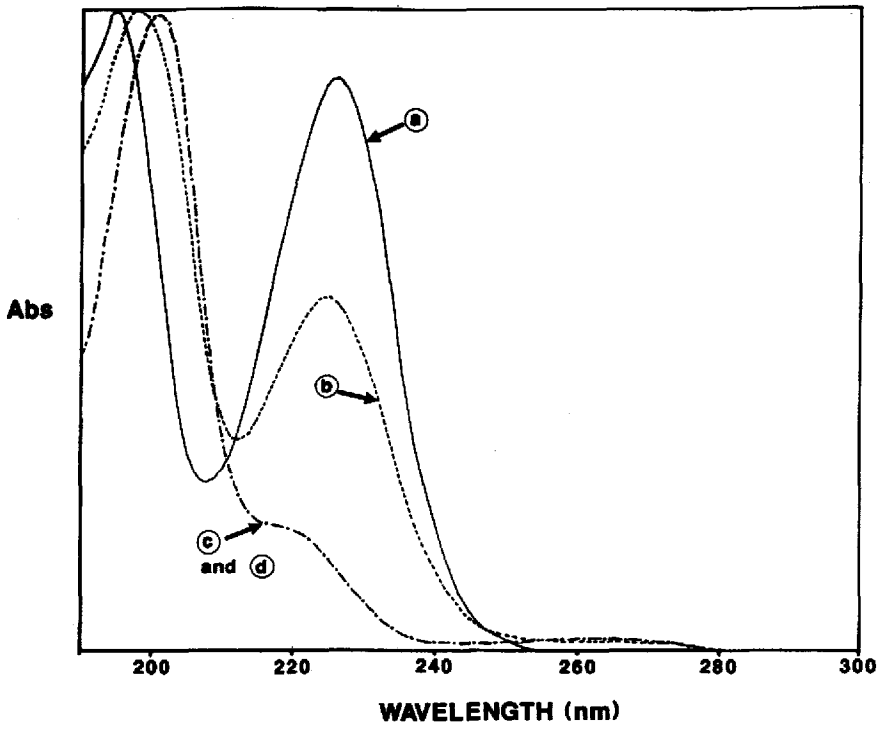

Fig. 4. UV spectra of (a) sodium iodide, (b) gallamine triethiodide, (c) gallamine base and (d) gallamine triethylperchlorate (measured on peak 4 by diode array detection).

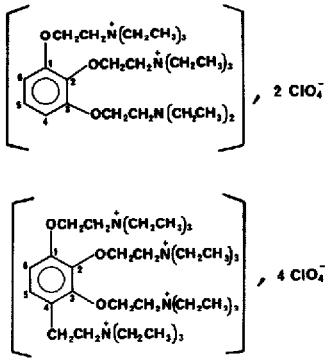

Fig. 5. Structures of (a) impurity 3 and (b) impurity 5.

4 when perchlorate replaces iodide on gallamine (Fig. 4d), is identical with that of gallamine base (Fig. 4c). Its maximum at 200 nm is the optimum detection wavelength (note that we observe a blue shift of about 3 nm for that maximum in the case of gallamine triethiodide. This is due to the merging of iodide and gallamine absorption bands).

In the chromatogram obtained, the iodide to gallamine peak-area ratio depends strongly on detection wavelength (Table I).

When the detection wavelength is set at 230 nm, the peak of gallamine is seven times smaller than that of iodide and this may induce chromatographic errors.

The difficulties in recent studies[5,6] in determining gallamine triethiodide by HPLC and in explaining the retention mechanisms can be understood if we assume that the compound determined was iodide instead of gallamine. In fact, in both instances, the detection wavelength was 230 and 229 nm and gallamine could not be detected.

Quantitative study

Calibration and reproducibility. Based on an acceptable signal-to-noise ratio of 3:1, the detection limit of gallamine at 200 nm is 35 ng/ml of gallamine triethiodide in the injection solution, which corresponds to 0.7 ng injected.

HPLC calibration has been performed on gallamine itself (peak 4) and on iodide. The concentrations of the injected gallamine triethiodide varied from 0.5 to

TABLE I

INFLUENCE OF THE DETECTION WAVELENGTH ON THE AREAS OF IODIDE AND GALLAMINE PEAKS

Parameter	*Wavelength (nm)*			
	200	*210*	*220*	*230*
Iodide peak area	825	339	644	817
Gallamine peak area	1114	609	236	117
Iodide to gallamine peaks-area ratio	0.74	0.56	2.73	6.98

TABLE II

ANALYSIS OF DATA FOR THE REPRODUCIBILITY OF THE ASSAY OF GALLAMINE AND IODIDE

Concentration (μg/ml)	*n*	*Iodide*		*Gallamine*	
		Mean peak area	*C.V.* (%)*	*Mean peak area*	*C.V. (%)*
151.9	15	6487	0.36	8748	0.19
0.50	15	29.6	5.14	29.18	1.65

* Coefficient of variation.

500 μg/ml. The correlation coefficient for gallamine of 0.9997 ($n = 58$) indicates excellent linearity for gallamine triethiodide concentrations between 0.5 and 250 μg/ml.

The same calculations were also done with iodide. The linearity is not as good as with gallamine and the correlation coefficient of 0.9995 ($n = 30$) indicates good linearity but in a more restricted concentration range (7–75 μg/ml). This is mainly caused by the low retention of iodide; peak broadening is weak and UV saturation appears at lower concentrations. On the other hand, at low iodide concentrations, a peak due to the solvent front disturbs the iodide quantification. Consequently, even though this method allows a good evaluation of iodide concentration, it is less precise than a volumetric method of quantification.

The accuracy of the method is shown in Table II. Excellent reproducibility is obtained, even at low concentrations, except for iodide which is difficult to measure for concentrations lower than 15 μg/ml.

We checked the stability of the gallamine triethiodide solution: after 1 week at room temperature, the variations in the iodide and gallamine peak area were not significant (Table III).

Quantification of impurities. The precise measurement of the response coefficients of the impurities, *i.e.*, gallamine base and impurities 3 and 5, requires large amounts of product. However, owing to substitution of perchlorate for iodide in the mobile phase, these impurities contain the same chromophore; all their UV spectra

TABLE III

ANALYSIS OF DATA FOR THE STABILITY OF GALLAMINE TRIETHIODIDE IN THE INJECTION SOLUTION (CONCENTRATION 151.9 μg/ml)

Time (days)	*n*	*Iodide*		*Gallamine*	
		Mean peak area	*C.V. (%)*	*Mean peak area*	*C.V. (%)*
0	15	6487	0.36	8748	0.19
1	4	6508	0.49	8735	0.04
2	4	6483	0.30	8760	0.21
5	4	6474	1.08	8729	0.14
7	4	6542	0.38	8747	0.12

are therefore nearly identical (similar to that of 1,2,3-trimethoxybenzene). Consequently, to a first approximation, their molar absorptivities are identical and the response coefficients to apply to the chromatographic results are roughly proportional to the molecular weight of the impurity.

We tested this hypothesis and found that the gallamine base response coefficient is 0.475 times that of gallamine triethiodide. This agrees well with the gallamine base to gallamine triethiodide molecular weight ratio (0.47).

TLC: Results and comparison with HPLC

Using TLC, the chloride ions, which are very concentrated in the mobile phase, immediately form ion pairs with gallamine, and iodide is eluted in the solvent front. This type of TLC system, in which counter ions such as Cl^-, Br^-, I^- or ClO_4^- are used with quaternary ammonium- or protonated amine-containing solutes has been described[30–32]. The retention mechanism is identical with that described above for silica.

Fig. 6 shows a thin-layer chromatogram of some gallamine batches. Five gallamine triethiodide batches of three different origins were applied to the plate (spots a–e) in addition to gallamine base (spot f) and a mixture of gallamine base, gallamine triethiodide and the two identified impurities (spot g). The R_F valves of gallamine base, impurity 3, gallamine triethiodide and impurity 5 are 0.79, 0.63, 0.53 and 0.39, respectively. For the two gallamine triethiodide batches spotted at a and b (Fig. 6) we observe another unknown impurity with $R_F = 0.18$.

To compare the HPLC results with those obtained by TLC, we injected the same gallamine batches into the HPLC system (Fig. 7). It was necessary to use a

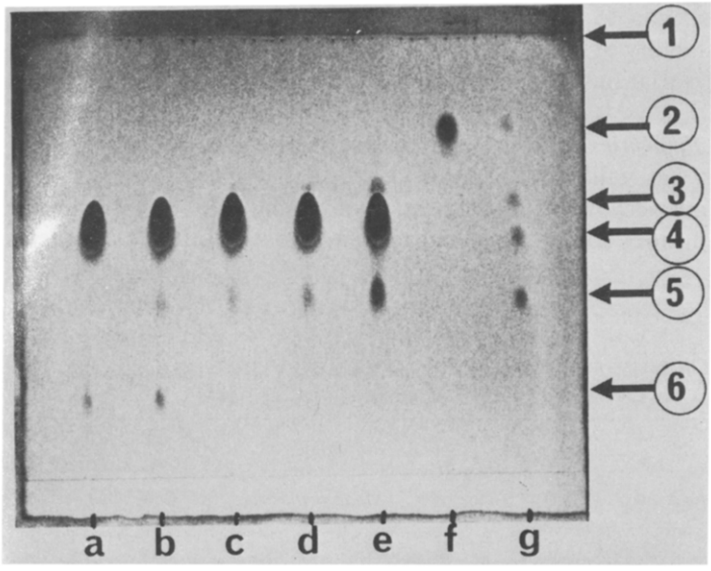

Fig. 6. Thin-layer chromatogram of some gallamine batches. Chromatographic conditions as described under Experimental. Solutes injected: (a–e) gallamine triethiodide batches; (f) gallamine base batch; (g) mixture of gallamine base, gallamine triethiodide and impurities 3 and 5. Spot identification: (1) chloride; (2) gallamine base; (3) impurity 3; (4) gallamine triethylchloride; (5) impurity 5; (6) unidentified.

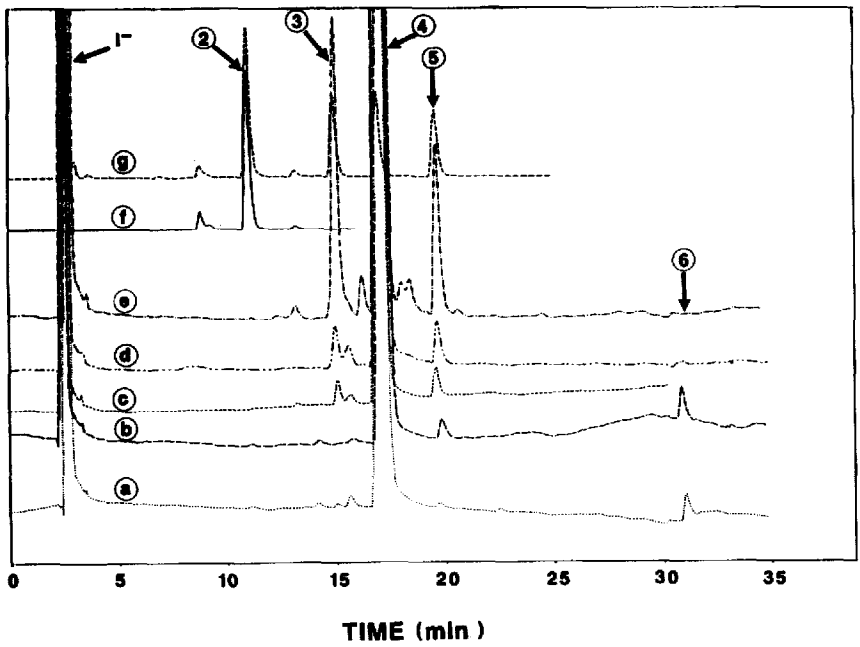

Fig. 7. Chromatograms of gallamine batches obtained with an elution gradient. Column and detection as in Fig. 2a; flow-rate, 1 ml/min. Mobile phase: the sodium perchlorate concentration was kept at 0.1 *M* and the water solution was buffered at pH 3; at time zero the composition was water–acetonitrile (76:24, v/v), changed linearly to (62:38, v/v) at 20 min and (54:24, v/v) at 25 min. (a–e) Gallamine triethiodide batches; (f) gallamine base; (g) synthetic mixture of gallamine base, gallamine triethiodide and impurities 3 and 5. Solutes: (2) gallamine base; (3) impurity 3; (4) gallamine triethylperchlorate; (5) impurity 5; (6) unknown impurity.

gradient to elute the impurity which may correspond to that revealed in TLC (R_F = 0.18). We observed perfect agreement between the impurities detected in HPLC and in TLC. Even though the new impurity has not yet been identified, and the detection methods used for HPLC and TLC are different (the spray reagent reveals the nitrogen-containing compounds), the new HPLC impurity (peak 6) probably corresponds to that marked 6 (R_F = 0.18) in TLC.

The elution order of all the gallamine impurities was the same in all the TLC and HPLC systems. This similarity between the elution order on octadecyl-bonded silica and on bare silica with an acidic water–organic solvent mobile phase and a counter ion such as perchlorate has already been observed at the beginning of the study.

CONCLUSION

An HPLC method for the determination of gallamine that gives precise and reproducible results has been developed. It was used on a semi-preparative scale to isolate and determine the structure of the two main impurities present in batches of gallamine. They can be quantified by this method as good estimates of their response coefficients compared with that of gallamine are given. The results obtained using a

TLC method for the examination of gallamine triethiodide are consistent with those obtained by HPLC.

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REFERENCES

1 S. Agonston, G. A. Vermeer, U. W. Kersten and A. H. Scaf, *Br. J. Anaesth.*, 50 (1978) 345.
2 M. I. Ramzan, E. J. Triggs and C. A. Shanks, *Eur. J. Clin. Pharmacol.*, 17 (1980) 135.
3 M. I. Ramzan, E. J. Triggs and C. A. Shanks, *Eur. J. Clin. Pharmacol.*, 17 (1980) 145.
4 M. I. Ramzan, E. J. Triggs and C. A. Shanks, *Eur. J. Clin. Pharmacol.*, 17 (1981) 141.
5 M. J. Shao, K. D. Fallon, S. N. Khalil and E. Abouleish, *J. Chromatogr.*, 345 (1985) 184.
6 I. M. Ramzan, *J. Chromatogr.*, 417 (1987) 428.
7 M. Beaumont, unpublished results.
8 G. Schill, *Nat. Bur. Stand. (U.S.) Spec. Publ.*, No. 519 (1979) 509.
9 G. Schill, *Acta Pharm. Fenn.*, 90 (1981) 43.
10 S. H. Hansen, P. Helboe and M. Thomsen, *J. Chromatogr.*, 409 (1987) 71.
11 W. E. Rudzinski, D. Benett and V. Garica, *J. Liq. Chromatogr.*, 5 (1982) 1295.
12 K.-G. Wahlund and S. Sokolowski, *J. Chromatogr.*, 151 (1978) 299.
13 F. P. Schmiidtchen and H. Oswald, *J. Liq. Chromatogr.*, 9 (1986) 993.
14 *Supelco HPLC Reporter*, Vol. 3, No. 2, Supelco, Bellefonte, PA, 1981.
15 J. A. de Schutter and P. de Moerloose, *J. Chromatogr.*, 437 (1988) 83.
16 M. G. M. de Ruyter, R. Cronnelly and N. Castagnoli, Jr., *J. Chromatogr.*, 183 (1980) 193.
17 M. Wolff, E. M. Winkler, D. Kersten and B. Goeber, *Pharmazie*, 40 (1985) 624.
18 B. A. Persson, S. O. Jansson, M. L. Johansson and P. O. Lagerstrom, *J. Chromatogr.*, 316 (1984) 291.
19 M. J. M. Wells, *J. Liq. Chromatogr.*, 5 (1982) 2293.
20 J. S. Kiel, S. L. Morgan and R. K. Abramson, *J. Chromatogr.*, 320 (1985) 313.
21 S. L. Abidi, *J. Chromatogr.*, 324 (1985) 209.
22 P. A. Mourier, *Analusis*, 17 (1989) 67.
23 J. E. Greving, H. Bouman, J. H. G. Jonkman, H. G. M. Westenberg and R. A. de Zeeuw, *J. Chromatogr.*, 186 (1979) 683.
24 J. H. Knox and R. A. Hartwick, *J. Chromatogr.*, 204 (1981) 3.
25 R. B. Taylor, R. Reid and C. T. Hung, *J. Chromatogr.*, 316 (1984) 279.
26 R. H. A. Sorrel and A. Hulshoff, *Adv. Chromatogr.*, 21 (1983) 87.
27 W. R. Melander and S. Horváth, in M. T. W. Hearn (Editor), *Ion-Pair Chromatography*, Marcel Dekker, New York, 1985, pp. 27–75.
28 A. Bartha, H. A. H. Billiet, L. de Galan and G. Vigh, *J. Chromatogr.*, 291 (1984) 91.
29 A. Bartha, G. Vigh, H. A. H. Billiet and L. de Galan, *J. Chromatogr.*, 303 (1984) 29.
30 R. Giebelmann, *Pharmazie*, 40 (1985) 299.
31 R. Giebelmann, *Pharmazie*, 36 (1981) 857.
32 R. Giebelmann, *Pharmazie*, 40 (1985) 108.