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Simple procedure involving derivatisation and thin-layer chromatography for the estimation of trace levels of halogenated alkylamines and their hydrolysis products in drug substances and formulations

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ABSTRACT

Thin-layer chromatographic methods are described for the assessment of residues of aminoalcohols in promethazine, gallamine and mepyramine (pyrilamine) injections using chromogenic/fluorogenic reagents. Detection limits of 8 ppm have been established for 2-dimethylaminopropan-1-ol and 1-dimethylaminopropan-2-ol in promethazine injection, 7 ppm for 2-diethylaminoethanol in gallamine injection and 1 ppm for 2-dimethylaminoethanol in mepyramine injection. These methods enable the assessment of residues of β-chloroalkylamine alkylating agents. The simplicity and sensitivity of these methods compare favourably with the few literature methods and offer scope for adaptation for other β-chloroalkylamines in basic drugs.

INTRODUCTION

Halogenated alkylamines are widely used throughout the pharmaceutical industry as alkylating agents in the manufacture of drug substances. Their use frequently occurs at an early stage in the manufacturing route, allowing subsequent stages for unreacted residues or their hydrolysis product, the aminoalcohol, to be removed by recrystallisation, extraction etc.

The chemistry of these simple molecules is relatively complex and received much concerted effort in the late 1940s and early 1950s [1–9]. From these studies, which mainly concerned their reaction kinetics, it can be inferred that: (i) in alkaline solution they are prone to intramolecular cyclisation with the rate of reaction dependent on the size of the ring formed; such cyclisation reactions occur rapidly even at ambient temperature (half-lives typically 1–10 min), (ii) although theoretically so, these cyclisations are rarely reversible because the competing hydrolysis reaction causes ring fission to the corresponding aminoalcohol.

The lowest member of the homologous series of such alkylating agents, 2-chloroethylamine, is extremely unstable and the alkylating agent of choice is

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normally the intramolecular cyclisation product, aziridine. Analytical methods have been reported for aziridine using derivatisation/spectrophotometry [10–12]; aziridine and its hydrolysis product, ethanolamine, can be analysed by ligand exchange chromatography [13]. Residues of 2-chloroethylamine and aziridine have recently been analysed in pharmaceuticals [14] by derivatisation gas chromatography.

Dialkylaminoethyl and propyl halides are more frequently employed as alkylating agents in the pharmaceutical industry but analytical procedures for their estimation have not been reported. Cyclisation of these dialkylaminoalkyl halides yields quaternary aziridinium ions which readily hydrolyse in solution to give tertiary aminoalcohols (Fig. 1).

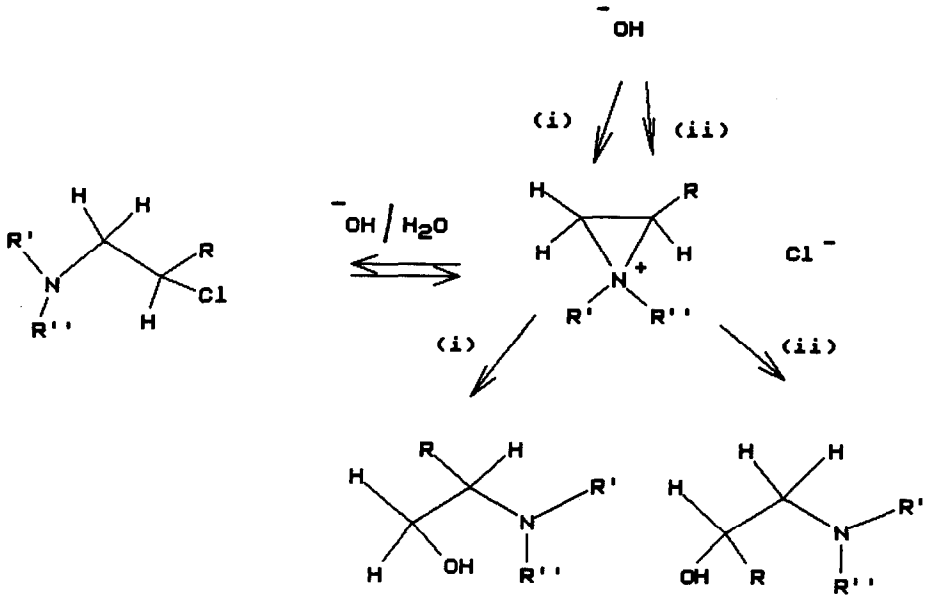

Fig. 1. Hydrolysis of β-chloroalkylamines to aminoalcohols. Key: R, R′, R″ = CH_3 (promethazine synthesis); R = H, R′, R″ = C_2H_5 (gallamine synthesis); R = H, R′,R″ = CH_3 (mepyramine synthesis).

The aziridinium ion derived from intramolecular cyclisation of 1-dimethylamino-2-chloropropane can yield two isomeric aminoalcohols on cleavage of the intermediate aziridinium ion, although attack at the least hindered carbon atom (route *i*), yielding 2-dimethylaminopropan-1-ol is favoured.

The derivatisation reaction schemes for the aminoalcohols are shown in Fig. 2.

This paper describes methods for the determination of trace amounts of the pharmaceutically important alkylating agents, 1-dimethylamino-2-chloroethane, 1-diethylamino-2-chloroethane and 1-dimethylamino-2-chloropropane via their corresponding aminoalcohols.

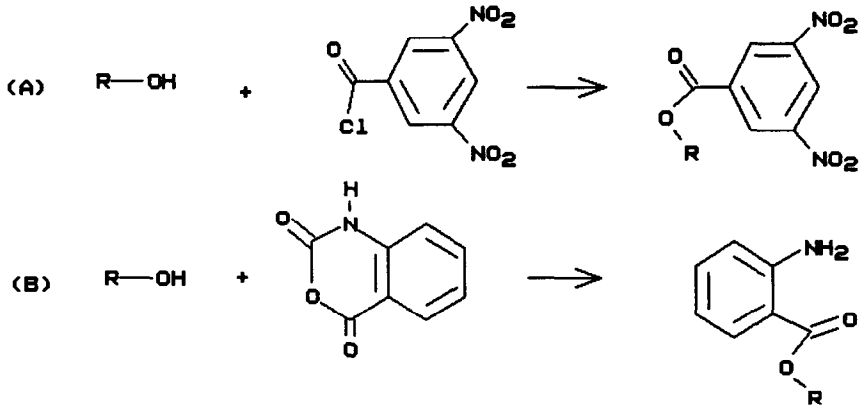

Fig. 2. Reaction schemes for derivatisation reactions. (A) Promethazine injection BP and gallamine injection BP; (B) mepyramine injection. R–OH represents the relevant aminoalcohol for each product.

EXPERIMENTAL

Samples

Samples of promethazine injection BP (British Pharmacopeia) [Phenergan injection 2.5% (w/v)], gallamine injection BP [Flaxedil injection 4.0% (w/v)] and mepyramine (pyrilamine) injection 2.5% (w/v) (Anthisan injection) were obtained from in-house stored check samples of production batches.

Chemicals

Aminoalcohols, aminoalcohol hydrochlorides, 3,5-dinitrobenzoyl chloride, 3,5-dinitrobenzoic acid and isatoic anhydride were all obtained from Aldrich (Gillingham, U.K.). 2-Dimethylaminopropan-1-ol was obtained from the Alfred Bader Library of Rare Chemicals (Aldrich).

Potassium carbonate (anhydrous) and potassium bicarbonate (anhydrous) were both Pronalys grade analytical reagents from the May & Baker range of laboratory chemicals (Rhône-Poulenc, Manchester, U.K.).

All other chemicals were of general reagent grade quality from May & Baker.

Apparatus

Octadecylsilyl (C_{18}) and silica sample preparation cartridges are part of the Sep-Pak range manufactured by Waters Associates and were obtained in the U.K. from Phase Separations (Queensferry, U.K.). Thin-layer chromatographic (TLC) plates, 20 × 20 cm, silica-gel GF_{254}, 0.25 mm thick manufactured by Merck were obtained from BDH (Dagenham, U.K.). These plates were activated for 1 h at 105°C before use. All other equipment is that generally found in an analytical chemistry laboratory.

Sample preparation —Promethazine injection BP

Condition a C_{18} Sep-Pak with 5 ml methanol followed by 5 ml deionised water. Attach the cartridge to a 20-ml glass syringe. Transfer 5 ml of injection solution into

the barrel of the glass syringe. Using the syringe plunger, elute the sample through the cartridge, collecting the eluate in a 100-ml amber separator containing 5 ml of 1 *M* hydrochloric acid solution. Elute the cartridge with 2 × 5 ml water, collecting the eluates in the separator. Discard the cartridge.

Extract the acidified eluate with 6 × 20 ml dichloromethane, shaking each aliquot for approximately 1 min. Discard the dichloromethane extracts and add 10 ml of 2.5 *M* sodium hydroxide solution. Transfer the solution to the barrel of a 20-ml glass syringe fitted with a new, conditioned C_{18} Sep-Pak cartridge. Collect the eluate in a second 100-ml amber separator. Elute the cartridge with 2 × 5 ml water, collecting the eluates in the separator. Discard the cartridge. Extract the solution with 4 × 5 ml dichloromethane and run each aliquot through anhydrous sodium sulphate into a 50-ml round-bottomed flask containing a solution of approximately 5 mg of 3,5-dinitrobenzoic acid in 1 ml diethyl ether[a].

Evaporate the dried dichloromethane extracts to dryness at ambient temperature using a rotary evaporator. Re-dissolve the residue in *ca.* 1 ml diethyl ether and add 10 mg of 3,5-dinitrobenzoyl chloride. Add 2 μl triethylamine as catalyst and stopper the tube to permit the derivatisation to proceed.

After 30 min, add 1 ml of 1.25 *M* (5%, w/v) sodium hydroxide solution, stopper and shake for approximately 1 min to destroy excess derivatising agent. Transfer the ether phase, using a Pasteur pipette, to a 5-ml pear-shaped flask. Repeat the extraction with a further 1 ml aliquot of ether, transferring the ether phase to the pear-shaped flask. Evaporate the ethereal solution to dryness and re-dissolve the residue in 100 μl of chloroform. The sample solution is now ready for spotting onto a TLC chromatoplate.

To prepare a "spiked" sample, transfer, by microsyringe, 10 μl *each* of 1-dimethylaminopropan-2-ol and 2-dimethylaminopropan-1-ol into a 100-ml measuring cylinder containing 100 ml of deionised water. Stopper and shake thoroughly. Pipette 10 ml into a second 100-ml measuring cylinder, dilute to volume with water and mix thoroughly ("spiking" solution). Carry out the extraction procedure as described above after adding 1 ml of "spiking" solution to the 5 ml bulked injection solution at the first stage of the procedure.

Sample preparation —Gallamine injection BP

The procedure for the sample solution is as described above for promethazine injection **BP** with the following amendment: a 4-ml sample of product is taken, equivalent to 160 mg of gallamine triethiodide contained in two 2-ml ampoules.

To prepare a "spiked" sample, transfer, by microsyringe, 10 μl of 2-diethylaminoethanol into a 100-ml measuring cylinder containing 100 ml of deionised water. Stopper and shake thoroughly. Pipette 10 ml into a second 100-ml measuring cylinder, dilute to volume with water and mix thoroughly ("spiking" solution). Carry out the extraction procedure as described above for promethazine injection **BP** after adding 1 ml of "spiking" solution to the 4 ml bulked injection solution at the first stage of the procedure.

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[a] The purpose of adding 3,5-dinitrobenzoic acid to the eluate prior to evaporation is to minimise evaporative loss of aminoalcohols known to otherwise occur.

Sample preparation —Mepyramine injection

Transfer 6 ml of injection solution into a 25-ml separator containing 2 ml of 2 *M* potassium carbonate solution. Extract the resulting mepyramine base suspension with 4 × 5 ml aliquots of dichloromethane, (1 min shaking for each aliquot). Discard the dichloromethane extracts and transfer the aqueous phase to a 25-ml measuring cylinder. Wash the separator with 1 ml of 2 *M* potassium hydrogen carbonate solution followed by 1 ml of deionised water and add both to the measuring cylinder.

Dissolve 50 mg isatoic anhydride in 5 ml acetonitrile in a sample tube then add it to the aqueous extract in the measuring cylinder; mix for about 30 s then heat at 75°C for 15 min in order to complete derivatisation.

Allow the mixture to cool to ambient temperature, add about 2 ml of acetonitrile and shake the cylinder for about 30 s. The upper (water in acetonitrile) layer is removed and introduced into a 100-ml round-bottomed flask. The extraction is repeated with a further 2 × 5 ml aliquots of acetonitrile which are combined in the round-bottomed flask. Evaporate the contents of the flask to dryness under vacuum at about 40°C. (*Care*: bumping may occur in the initial stages of evaporation.) The residue is triturated with about 15 ml dichloromethane and the suspension transferred to a 100-ml separator containing 50 ml of 0.5 *M* sodium hydroxide solution. Shake the contents of the separator for about 1 min and transfer the lower organic layer into a 100-ml round-bottomed flask via a plug of anhydrous sodium sulphate. Re-extract with a further 15 ml aliquot of dichloromethane. Finally, wash the sodium sulphate with about 5 ml of dichloromethane adding this to the combined extracts. Concentrate the solution to about 2 ml by evaporation under vacuum. Wash a silica sample preparation cartridge with 10 ml of dichloromethane. Transfer the concentrated dichloromethane extract into the barrel of a 20-ml glass syringe attached to the cartridge. Transfer the sample onto the cartridge by means of the syringe plunger and discard the eluate. Wash the round-bottomed flask and syringe barrel with further aliquots of dichloromethane (up to 10 ml) and elute the washings through the cartridge. Discard the eluates. Elute the cartridge with 10 ml dichloromethane followed by 10 ml acetonitrile and discard the eluates. Elute the cartridge with 10 ml methanol and collect the eluate in a 100-ml round-bottomed flask.

Evaporate the methanol eluate to dryness under vacuum, re-dissolve the residue in about 1 ml of dichloromethane and transfer the solution to a 5-ml pear-shaped flask. Evaporate to dryness under vacuum and repeat the procedure by introducing a further 1 ml aliquot of dichloromethane used to wash the round-bottomed flask.

Re-dissolve the residue in 100 μl of dichloromethane. The sample solution is now ready for spotting onto a chromatoplate.

To prepare a "spiked" solution, transfer 5 μl of 2-dimethylaminoethanol into a 100-ml measuring cylinder containing 100 ml water. Stopper and shake thoroughly, ("spiking" solution). Carry out the extraction procedure as described above after adding 100 μl of "spiking" solution to the 6 ml bulked injection solution at the first stage of the procedure.

Adaptation to drug substances and synthetic intermediates

Residual levels of β-chloroalkylamines in drug substances or synthetic intermediates may be calculated indirectly from experimentally determined aminoalcohol contents of autoclaved injection solutions, as described earlier. However, these

experimental methods may be readily adapted to provide direct assessment of β-chloroalkylamine residues. Hydrolysis of β-chloroalkylamines in alkaline solution (pH >12) at 75°C for 15 min has been shown to produce quantitative yields of the corresponding aminoalcohols.

Adaptation of the methods described for Phenergan injection 2.5% (w/v) and Flaxedil injection 4.0% (w/v) for the estimation of 1-dimethylamino-2-chloropropane and 1-diethylamino-2-chloroethane respectively has been achieved by introducing an hydrolysis step after the addition of 10 ml of 2.5 *M* sodium hydroxide. Aliquots of promethazine hydrochloride, 125 mg or gallamine triethiodide, 160 mg are dissolved in 5 ml of water and are analysed according to the respective methods for the injection solutions with the additional hydrolysis steps.

Analysis of β-chloroalkylamine residues in promethazine *base* may be accomplished by dissolving 110 mg of the drug substance in 4 ml of 0.1 *M* hydrochloric acid rather than water.

The method for Anthisan injection is also readily adapted for estimating residues of 1-dimethylamino-2-chloroethane in mepyramine maleate drug substance. Dissolve 150 mg of mepyramine maleate in 6 ml of water in a 50-ml measuring cylinder. Add 2 ml of 2 *M* potassium carbonate solution and heat at 75°C for 15 min. Allow to cool and extract with 4 × 5 ml portions of dichloromethane as described above.

Thin-layer chromatography

Sample spots (40 μl and 20 μl) and "spiked" sample spots (20, 10, 5, 2 and 1 μl) are spotted onto activated silica-gel layers (0.25 mm thickness). The plates are eluted for 10 cm in 100% acetone (promethazine and gallamine) or 100% methanol (mepyramine). The TLC tanks are saturated with their respective solvents.

Detection is made under 254 nm irradiation (aminoalcohol residue derivatives from promethazine and gallamine) or under 366 nm irradiation (aminoalcohol residue derivative from mepyramine). The estimation of aminoalcohol content is achieved by calculating the proportion of the weight of aminoalcohol detected in sample chromatograms to the weight of drug substance taken and expressing the result as ppm. The β-chloroalkylamine content may then be calculated from the relative molecular weights of β-chloroalkylamine and aminoalcohol. In the case of injection solutions, a correction should be made for the expected degree of hydrolysis of β-chloroalkylamines during autoclaving (see Results and Discussion).

RESULTS AND DISCUSSION

The aminoalcohols could not all be determined using the same method since 2-dimethylaminoethanol remained preferentially in an alkaline aqueous phase and was also lost during evaporation in the final stage of the concentration process.

A possible alternative method for 2-dimethylaminoethanol residues in Anthisan injection was that previouly reported for traces of glycols, polyols and hydroxylamines in aqueous media [15]. It has been shown that these residues can be isolated by the use of C_{18} sample preparation cartridges, dried with nitrogen and subsequently desorbed with a polar organic solvent such as acetonitrile. However, although this method could be applied successfully to traces of 2-dimethylaminoethanol in water, it failed to retain the aminoalcohol in the presence of mepyramine maleate drug substance. The method

finally developed involved the minimum of sample handling and permitted the derivatisation to take place in an aqueous/organic medium.

The 3,5-dinitrobenzoate ester derivatives of the aminoalcohol residues from Phenergan and Flaxedil injections were not very stable and readily underwent hydrolysis or solvolysis in the presence of protic solvents. Further investigation confirmed that isatoic anhydride would readily form an anthraniloate ester with 2-dimethylaminoethanol with the advantage that it was effective in aqueous/organic solutions [16,17], yielding a stable, highly fluorescent derivative. This reagent was thus adopted for use with samples of Anthisan injection.

The chromatographic properties of the various derivatives were investigated using TLC with 100% acetone for elution of the 3,5-dinitrobenzoate esters and 100% methanol for the anthraniloate ester. Linear intensity gradients for absorption at 254 nm against concentration were found for the 3,5-dinitrobenzoate esters of 1-dimethylaminopropan-2-ol, 2-dimethylaminopropan-1-ol and 2-diethylaminoethanol over the *apparent* range 0.4–3.2 μg with respect to the underivatised aminoalcohols (*i.e.* complete derivatisation cannot be assumed). A linear intensity gradient for fluorescence emission intensity was also found for 2-dimethylaminoethyl anthraniloate over the *apparent* range 0.05–1.0 μg with respect to the underivatised aminoalcohol.

When applied to solutions containing either promethazine hydrochloride or gallamine triethiodide, the procedure separated the 3,5-dinitrobenzoate esters of the aminoalcohols from excess reagent, 3,5-dinitrobenzoic acid (solvolysis by-product and also added to avoid evaporative loss of aminoalcohol) and from traces of co-extracted drug substance. Approximate R_F values are given in Table I. The TLC method for the anthraniloate ester of 2-dimethylaminoethanol enables separation of the derivative from excess reagent, anthranilic acid and from traces of co-extracted drug substance. Approximate R_F values are given in Table II.

All derivatives can be positively identified by spraying with Dragendorff's reagent [18], producing a red–brown colouration of the insoluble tertiary-amino iodo-bismuthate salt.

Because of the likelihood of traces of both the β-chloroalkylamine and the

TABLE I

APPROXIMATE R_F VALUES FOR COMPONENTS IN ACETONE (100%) MOBILE PHASE

Component	Approximate R_F
3,5-Dinitrobenzoate esters of:	
2-Dimethylaminopropan-1-ol	0.20
1-Dimethylaminopropan-2-ol	0.38
2-Diethylaminoethanol	0.32
3,5-Dinitrobenzoyl chloride (reagent)[a]	0.04–0.16 (streak)
3,5-Dinitrobenzoic acid[b]	0.04–0.19 (streak)
Promethazine	0.05–0.15 (streak)
Gallamine	baseline–0.11 (streak)

[a] Excess reagent is mostly destroyed during sample work-up.

[b] This component remains in an aqueous (discarded) phase during sample work-up.

TABLE II

APPROXIMATE R_F VALUES FOR COMPONENTS ELUTED IN METHANOL (100%) MOBILE PHASE

Component	Approximate R_F
2-Dimethylaminoethyl-anthraniloate	0.28
Isatoic anhydride (reagent)[a]	0.70
Anthranilic acid[b]	0.66
Mepyramine	0.13–0.25 (streak)

[a] Excess reagent is mostly destroyed during sample work-up.
[b] This component remains in an aqueous (discarded) phase during sample work-up.

corresponding aminoalcohol being present together in autoclaved injection solutions, simulated autoclaving experiments were carried out on samples of the parent β-chloroalkylamines for each product studied in order to assess the degree of hydrolysis to the corresponding aminoalcohols. Dilute stock solutions of the β-chloralkylamines were prepared in 0.01 *M* hydrochloric acid in order to inhibit hydrolysis prior to the autoclaving experiments. Table III contains details of autoclave conditions, weights of β-chloroalkylamine taken, the experimental pH values and the approximate degrees of conversion to aminoalcohols (by comparison with TLC spot intensities of derivatives from known amounts of aminoalcohols).

The different pHs chosen for simulated autoclave experiments reflect those extremes of pH commonly found in samples of injection solutions containing the drug substances.

The limits of detection for the aminoalcohols, combined with the observed degrees of hydrolysis of the β-chloroalkylamines enable the limits of detection for the β-chloroalkylamines (as hydrochlorides) in the drug substances to be calculated indirectly. These limits are given in Table IV.

TABLE III

APPROXIMATE PERCENTAGE CONVERSION OF β-CHLOROALKYLAMINES UNDER CONDITIONS SIMULATING THE PRODUCTION OF INJECTIONS

Autoclave conditions	Name and weight of β-chloroalkylamine	pH	Aminoalcohol(s) produced	Approx. conversion (%)
121°C/20 min	1-Dimethylamino-2-chloropropane (25 μg)	5.0	1-Dimethylaminopropan-2-ol 2-Dimethylaminopropan-1-ol	None detected 40
121°C/20 min	1-Dimethylamino-2-chloropropane (25 μg)	6.0	1-Dimethylaminopropan-2-ol 2-Dimethylaminopropan-1-ol	None detected 80
121°C/20 min	1-Diethylamino-2-chloroethane (14 μg)	5.5	2-Diethylaminoethanol	25
121°C/20 min	1-Diethylamino-2-chloroethane (13 μg)	7.5	2-Diethylaminoethanol	50
115°C/30 min	1-Dimethylamino-2-chloroethane (8 μg)	6.0	2-Dimethylaminoethanol	100

TABLE IV

LIMITS OF DETECTION FOR AMINOALCOHOLS IN INJECTION SOLUTIONS AND β-CHLOROALKYLAMINES IN DRUG SUBSTANCES

Injection	Drug substance	Limit of detection, ppm[a]	
		Aminoalcohol(s)	β-Chloroalkylamine (as hydrochloride)
Phenergan	Promethazine hydrochloride	8 (for each)	15–30 (for each)[b]
Flaxedil	Gallamine triethiodide	7	20–40[b]
Anthisan	Mepyramine maleate	1	1

[a] ppm with respect to the nominal content of drug substance.

[b] The range represents the uncertainty due to the effect of product pH upon the degree of hydrolysis of β-chloroalkylamine to aminoalcohol.

Samples of five batches of Phenergan injection manufactured between May 1985 and May 1987 contained no detectable trace (limit 8 ppm) of either 2-dimethylaminopropan-1-ol or 1-dimethylaminopropan-2-ol.

Samples of nine batches of Flaxedil injection manufactured between October 1984 and September 1987 contained no detectable trace (limit 7 ppm) of 2-diethylaminoethanol.

Samples of three batches of Anthisan injection manufactured in 1985 contained no detectable trace (limit 1 ppm) of 2-dimethylaminoethanol.

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