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Automated multiple development thin-layer chromatography for separation of opiate alkaloids and derivatives

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Abstract

There are three types of opiate alkaloids. First, the poppy alkaloids: morphine, codeine, thebaine, noscapine and papaverine; then, the semi-synthetic and synthetic derivatives used in therapy as antitussives and analgesics, such as pholcodine, ethylmorphine and dextromethorphan; at last narcotic compounds, diacetylmorphine (heroin) and opiates employed as substitutes in treatment of addiction: buprenorphine and methadone. For classical thin-layer chromatography (TLC) of opium alkaloids, it is necessary to use complex eluents with strong alkaline substances to obtain a clean separation between morphinan and isoquinoline compounds. This study purposes the planar chromatographic analysis of these substances by the automated multiple development (AMD) compared with results obtained by classical TLC method. The aim of this work was to achieve the best separation of these opiate alkaloids and derivatives by this modern technique of planar chromatography. The AMD system provided a clean separation for each of three opiates groups studied and the best results have been obtained with universal gradient: methanol 100, methanol–dichloromethane 50/50, dichloromethane 100, dichloromethane 100, hexane 100 for opium alkaloids and with gradient A: 5% of 28% ammonia in methanol 100, acetone 100, acetone 100, ethyl acetate–dichloromethane 50/50, dichloromethane 100 for antitussives and substitutes. Two reagents were used for the detection of alkaloids by spraying: Dragendorff and iodoplatinate reagents [17]. The detection limits with these two reagents were 1 μg for ethylmorphine, thebaine, papaverine, codeine, and 2 μg for morphine and noscapine and other alkaloids.

Keywords: Opiate alkaloids; Derivatives; Antitussives; Addiction drug; AMD

1. Introduction

Opiates are the most powerful known pain relievers, they are derivatives of opium and can produce euphoria but also they are used as analgesics. Opiates can be classified according to three series. The first one is constituted by the poppy alkaloids: morphine, codeine, thebaine, noscapine and papaverine (Fig. 1); the second category included mainly semi-synthetic derivatives of morphine or synthetic compounds are used in therapy as antitussives and analgesics: pholcodine, ethylmorphine (codethyline), dextromethorphan (Fig. 2); at last, the third class is composed of narcotic compounds: diacetylmorphine (heroin), and other opiates; they are employed as substitutes in treatment of addiction: buprenorphine, and methadone (Fig. 3).

Automated multiple development (AMD) is an instrumental technique of planar chromatography [1–4] which uses an eluent gradient starting from the most polar to the least polar [5]; the migration is performed by successive steps (15–25) and at each new development the proportions of the eluent constituents change; so the polarity is decreasing when the distance increases (Fig. 5).

Gradient development with linear eluotropic profile [6] leads to a band re-concentration improving the separation [1]. A successful separation depends mainly on the choice of the solvent components, optimisation of the shape of the gradient, the stepwise movement of the elution front [3,7] and the repeated developments increase the resolution [8].

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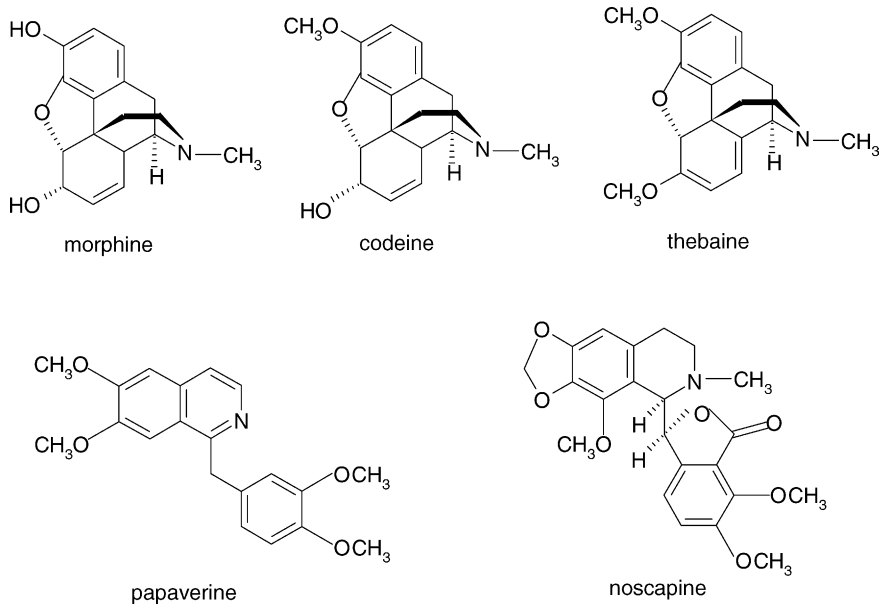

Fig. 1. Structure of opium alkaloids.

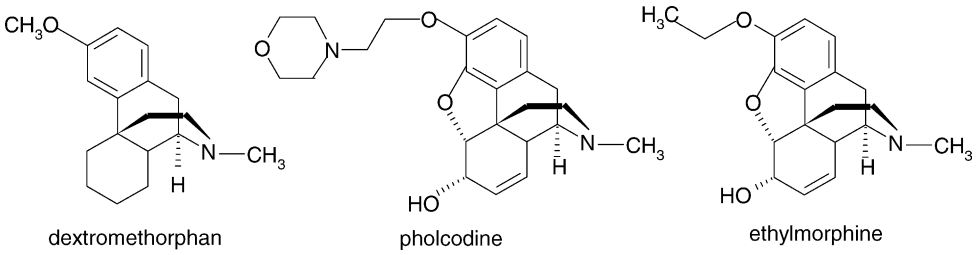

Fig. 2. Structure of antitussives.

This chromatographic technique is available for separation of plant extracts [9–16], mainly herbal drugs which present an interest with therapy activity and chemical substances belonging to various classes as: essential oils, alkaloids, resins, phenolic compounds. Besides, this method has permitted to analyse various chemical classes of alkaloids and phenolic compounds [10–12] with similar chemical properties and also isomeric position compounds [11]. In the field of detection of abuses, AMD was used with success for the chromatographic analysis of cannabinoids [13].

The aim of this work was to apply the performances of AMD to the separation of opium alkaloids, antitussives and narcotic compounds and to obtain a clean separation for each group studied.

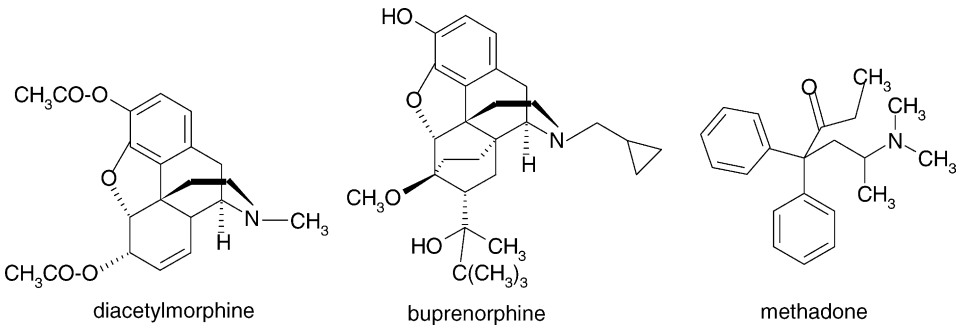

Fig. 3. Structure of derivatives and substitutes.

2. Experimental

2.1. Apparatus and materials

Chromatographic separations were performed using the AMD system (Camag, Muttenz, Switzerland).

Plates used were HPTLC silica gel F_{254} 10 cm × 20 cm on glass, layer thickness 0.1 mm, Article 11764, and HPTLC silica gel F_{254} 10 cm × 20 cm on glass, layer thickness 0.2 mm, Article 5642 (Merck, Darmstadt, Germany).

Samples were applied with a Linomat IV system (Camag, Muttenz, Switzerland).

All the solvents were analytical grade and purchased from Carlo Erba Reactifs (Val de Reuil, France). Before use, the solvents were filtrated through a 0.45 μm Millipore membrane (Millipore, Saint-Quentin, Yvelines, France) after sonication.

All the standards were commercially pure products.

2.2. Preparation of samples

All standard solutions were prepared by dissolving 10 mg of reference substance with 1 mL methanol.

The opium extract was obtained by shaking 2 g of raw opium powdered (180) according to *European Pharmacopoeia* (5.0) with 20 mL hydrochloride acid (0.1 M) during 5 min. After filtration, the acidic solution was alkalised by 28% ammonia until pH 10 and extracted 3 times with 10 mL of dichloromethane. The filtrate was evaporated to dryness and the residue solved in 2 mL methanol.

2.3. Application

Samples were applied as 20 mm bands and the volume was 3 μL for diacetylmorphine; 4 μL for morphine, thebaine, noscapine, papaverine, dextromethorphan, pholcodine and ethylmorphine; 5 μL for opium extract and codeine; 6 μL for buprenorphine; 7 μL for methadone.

2.4. Detection

Two reagents were used for the detection of alkaloids by spraying: Dragendorff according to Munier and Macheboeuf and iodoplatinate reagents [17].

The detection limits with these two reagents were 1 μg for ethylmorphine, thebaine, papaverine, codeine, and 2 μg for morphine and noscapine and other alkaloids.

3. Results and discussion

3.1. Separation of opiate alkaloids by classical TLC

The classical thin-layer chromatography analysis of opium alkaloids is not easy because these alkaloids belong to two different groups: morphinan (morphine, codeine and thebaine) and benzylisoquinoline (noscapine, papaverine) and their chromatographic behaviour is very different; the morphinan group presents low hR_F and isoquinoline group very high hR_F. For these reasons the authors use generally complex eluents, for example: diisopropyl ether–ethanol–diethylamine (97:2:1) [18], chloroform–ethanol–diethylamine (89:10.5:0.5) [19], toluene–ethyl acetate–diethylamine (70:20:10) [17] and *tert*-butanol, 28% ammonia–methanol–water (20:1:2:4) [20].

Sometimes, with complex eluents, the hR_F of some alkaloids are near or identical, and on the other hand, the presence of diethylamine brings about a difficult revelation because it is not easy to eliminate the amine from the chromatographic plate, so, the use of eluent with ammonia is better [21].

Among eluents without diethylamine, the *European Pharmacopoeia* uses toluene–acetone–ethanol–28% ammonia (40:40:6:2) for the separation of opium alkaloids. The best results were obtained from this eluent after changing slightly the solvent proportions: toluene–acetone–ethanol–28% ammonia (45:45:7:1) [10].

3.2. Separation by OPLC

In a previous work, OPLC had been used for the separation of opium alkaloids and analogues with only ethyl acetate as mobile phase and aluminium oxide as sorbent [22,23]. This is a significant improvement because usually in TLC and OPLC systems three or more components in the mobile phase are required. The use of a single solvent ensures good reproducibility of the method. The procedure is also interesting because the different compounds studied are cleanly separated [10].

3.3. Separation by AMD

In AMD, several parameters must be considered to obtain the best separation: choice of solvents, eluent gradient and number of steps.

In the first experimentation, the universal gradient: methanol 100, methanol, dichloromethane 50/50, dichloromethane 100, dichloromethane 100, and hexane 100 during 20 steps (Table 1) was used; the term universal characterizes a gradient which performs a separation of a mixture with a large polarity scale [9]. This gradient permitted a very clean separation of the major alkaloids from opium extract: morphine, codeine, thebaine, noscapine and papaverine. This one shows zones corresponding to these of the standard

Table 1
AMD universal gradient for opium alkaloids

Starting with step no.	1	2	6	11	16
Bottle no.	1	2	3	4	5
Methanol	100	50			
Dichloromethane		50	100		
Dichloromethane				100	
Hexane					100

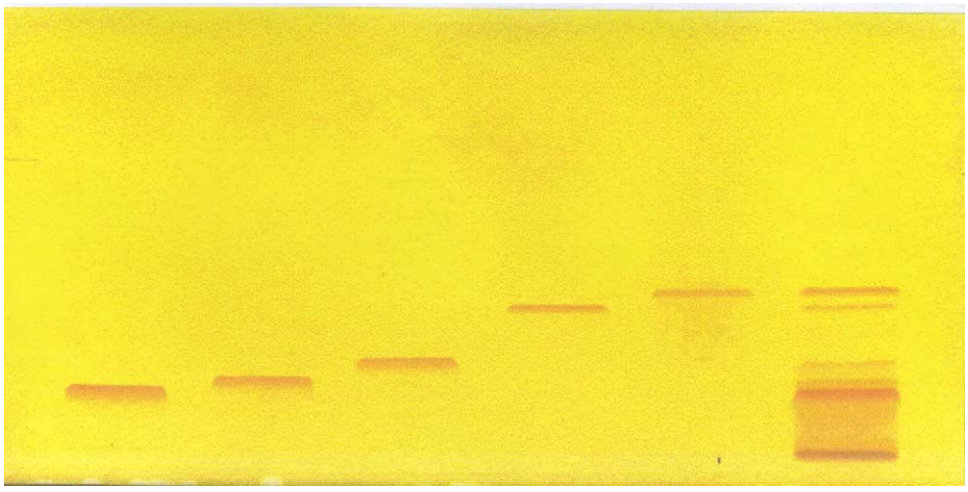

Fig. 4. HPTLC chromatogram by AMD of opium extract and standard alkaloids of opium: (1) morphine; (2) codeine; (3) thebaine; (4) papaverine; (5) noscapine; (6) opium extract; eluent used was universal gradient: methanol 100, methanol–dichloromethane 50/50, dichloromethane 100, dichloromethane 100, hexane 100; derivatization by Dragendorff reagent.

solutions, orange–red or red with dragendorff or other colours with iodoplatinate reagent's (Table 3; Fig. 4).

The other alkaloids studied (antitussives and derivatives) have not been able to be separated, therefore, hexane, no polar solvent, was deleted and replaced by dichloromethane. Some new gradients were established with: methanol, acetone, ethyl acetate and dichloromethane and, the best results were obtained with the eluent: methanol 100, methanol–acetone 50/50, acetone 100, ethyl acetate 100, ethyl acetate–dichloromethane 50/50, dichloromethane 100 for the separation of semi-synthetic and antitussives derivatives.

With this eluent, it was not possible to have a good separation of the other opiate derivatives because these

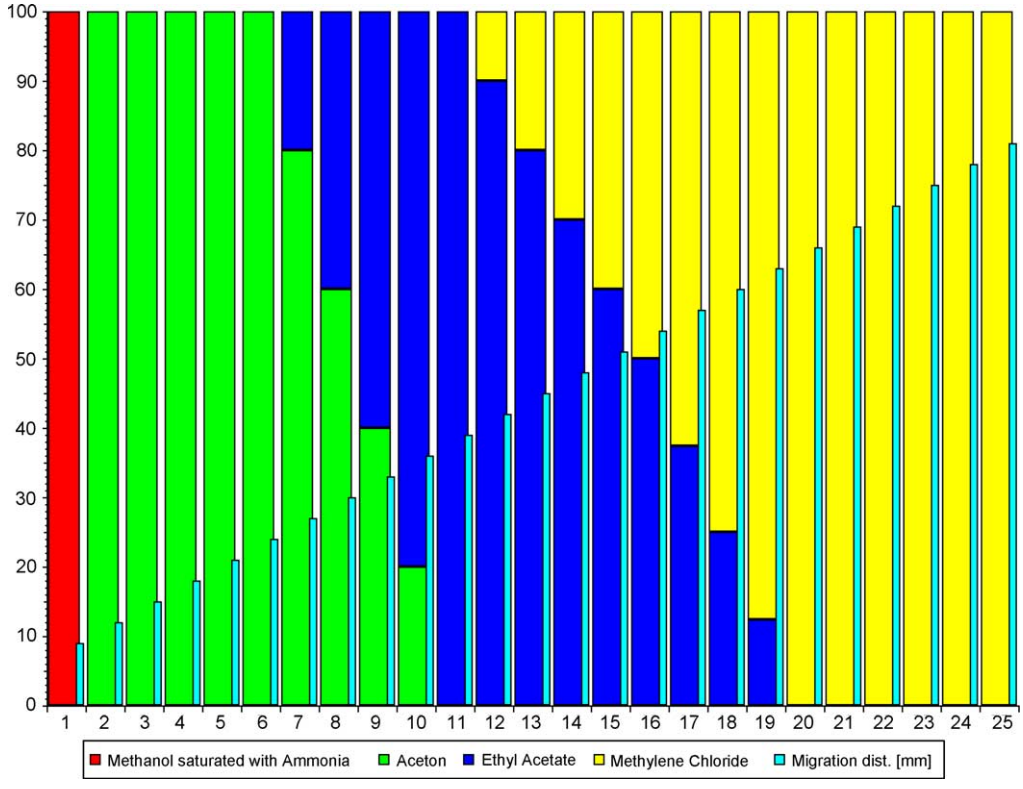

Fig. 5. AMD gradient for antitussivess, opiate derivatives and substitutes: This graphic which represent the solvent settings is slightly different with the composition of the solvent mixture in the tank, at each step. As the new solvent is mixed up with the remaining solvent from the previous step, there is for instance a little portion of the first polar solvent still down to the 10th step.

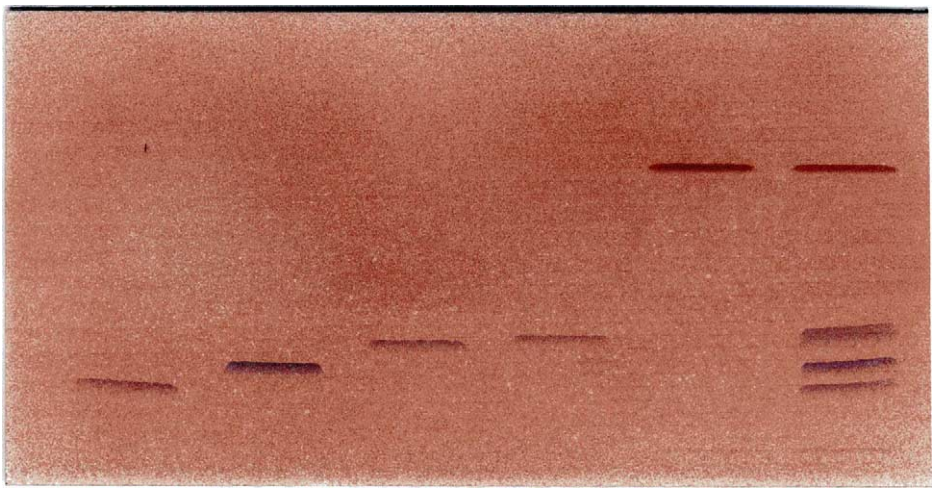

Fig. 6. HPTLC chromatogram by AMD of antitussives: (1) pholcodine; (2) dextromethorphan; (3) ethylmorphine; (4) codeine; (5) noscapine; (6) mixture of the five standards; gradient used was gradient A: 5% of 28% ammonia in methanol 100, acetone 100, acetone 100, ethyl acetate 100, ethyl acetate–dichloromethane 50/50, dichloromethane 100; derivatization by iodoplatinate of potassium.

Table 2
AMD gradient A for opiate antitussives and substitutes

Starting with step no.	1	2	6	11	16	20
Bottle no.	1	2	3	4	5	6
Methanol saturated ammonia	100					
Acetone		100	100			
Ethyl acetate				100	50	
Dichloromethane					50	100

compounds are more polar so, for antitussives and substitutes, the gradient used was: 5% of 28% ammonia in methanol 100, acetone 100, acetone 100, ethyl acetate 100, ethyl acetate–dichloromethane 50/50, dichloromethane 100 (gradient A, Table 2; Fig. 5).

With this eluent, a clean separation was obtained with antitussives (Fig. 6) and opiate derivatives and substitutes (Fig. 7); the hR_F values obtained by AMD are listed in Table 3.

AMD presents numerous advantages: a good resolution, a migration without oxidation because the chromatographic micro-chamber is saturated with methanol under a nitrogen atmosphere. The fully automated development of the plates (preconditioning time, automated eluent gradient, dry time) determines a good reproducibility of the analysis and the accurate mixture eluent for each step permit a sharper separation in well defined experimental conditions with no spot diffusion in the adsorbent and also reproducible hR_F values [5]. The slight diffusion observed in the low hR_F is decreasing until the top of the plate while in classical TLC and in OPLC techniques, the diffusion spot is increasing with the migration, this character is a limiting factor especially in the case of analysis by densitometry.

With this method, there is no manipulator effect and it is possible to operate automatically outside working hours without watch.

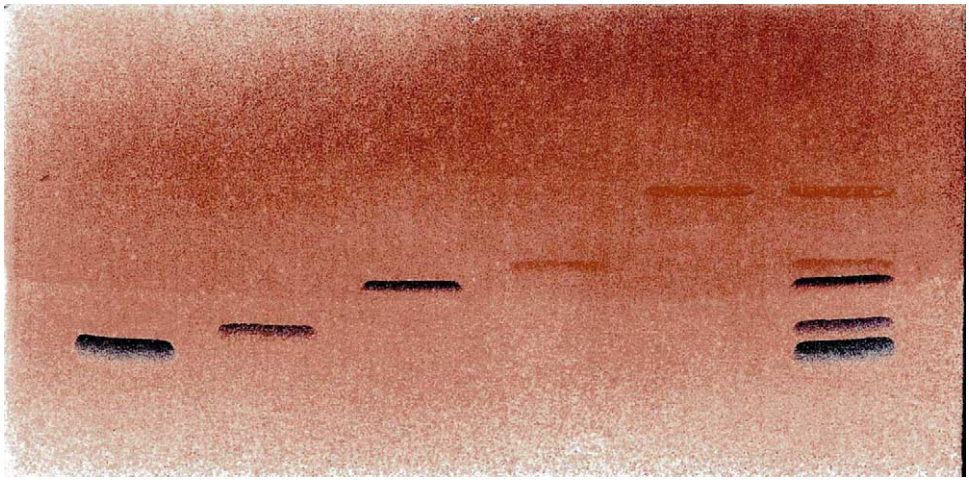

Fig. 7. HPTLC chromatogram by AMD of opiates, derivatives and substitutes: (1) morphine; (2) codeine; (3) diacetylmorphine; (4) methadone; (5) buprenorphine; (6) mixture of the five standards; gradient used was gradient A: 5% of 28% ammonia in methanol 100, acetone 100, acetone 100, ethyl acetate 100, ethyl acetate–dichloromethane 50/50, dichloromethane 100; derivatization by iodoplatinate of potassium.

Table 3
hR_F and colours with iodoplatinate reagent of opiate alkaloids, antitussives and substitutes

Compound	hR_F AMD	Colour with iodoplatinate
Opium alkaloids "universal gradient"		
Morphine	20	Deep blue
Codeine	25	Pink violet
Thebaine	32	Brown violet
Papaverine	52	Light pink
Noscapine	57	Pink brown
Opiates and analogues (gradient A) (iodoplatinate)		
Morphine	42	Deep blue
Codeine	45	Pink violet
Diacetylmorphine	64	Deep blue
Methadone	84	Pink violet
Buprenorphine	98	Pink violet
Antitussives (gradient A) (iodoplatinate)		
Pholcodine	23	Blue violet
Dextromethorphan	28	Blue violet
Ethylmorphine	35	Violet
Codeine	39	Pink violet
Noscapine	92	Pink brown

If the experimentation is realized with the regulation of the temperature, all the conditions are got together to increase the reproducibility in planar chromatography analysis.

AMD can have applications to control the quality of opium according to the norms required by the *Pharmacopoeia*. The best resolution and the lack of diffusion makes that AMD is the method which is the most available for densitometry and can be an alternative to isocratic chromatography described in many pharmacopoeias.

4. Conclusion

Planar chromatography is always a choice method for the analysis of natural products and medicinal plants. It has become a modern technique with the arrival of automated apparatus for the application of samples and automated development chambers (OPLC and AMD) which are available techniques particularly to separate constituents in crude extracts.

OPLC and AMD are more appropriate in many instances because these techniques give better reproducible hR_F values in well-defined experimental conditions.

AMD presents the best resolution, elution without oxidation, various possibilities of gradient and fully automated development of the plates. AMD permits the analysis on very small quantities and obtains sharper separations because the absence of diffusion in the adsorbent at the upper hR_F; this makes it a very interesting method for densitometry.

AMD has proved to be an efficient planar chromatographic technique that provides increased separation for compounds with neighbouring structures. Opium alkaloids, derivative compounds and also antitussives and substitutes are cleanly separated.

This AMD technique can be applied not only to the determination of poppy constituents but also in pharmaceutical analysis for antitussives, and above all in the field of abuses in toxicology.

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