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Normal- and bonded-phase liquid chromatography with photodiode array detection of maytansinoids

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Abstract

Maytansinoids obtained from *Maytenus* species are known as potential anti-cancer drugs. Investigation of new plant species for the presence of unknown maytansinoids as well as synthetic work requires a reliable analytical and semipreparative normal-phase separation which can distinguish between known and unknown compounds independently from retention times and which would be compatible to LC–MS interfacing for further studies. A thorough study of numerous maytansinoids on silica gel and on diol-, aminopropyl- and cyanopropyl-bonded phases, suggests a complex retention mechanism based on molecular size as well as on specific interaction with the stationary phase while limiting a selectivity effect of the mobile phase. Utilizing a Varian 9065 photodiode array detector a spectral library of the separated substances was generated. This library simplifies the identification of known compounds in a matrix of unknowns such as fractions of plant extracts even if baseline separation is not achieved. Unlike reversed-phase separations that have been described in the literature the presented method allows an easy sample recovery in a semipreparative separation.

Keywords: Stationary phases, LC; Mobile phase composition; Maytansinoids

1. Introduction

Maytansine (M1) is a potent antileukemic agent which was first isolated along with nine homologous maytansinoids by *Kupchan* and co-workers from *Maytenus* species [1]. Other maytansinoids have subsequently been isolated from plants of the Rhamnaceae [2] and Euphorbiaceae [3] families and from *Nocardia* cell cultures [4].

All maytansinoids have very similar structures as seen in Fig. 1. Molecular differences are usually found in the fatty acid moiety of the maytanside ester [5] at C3, which makes separation difficult. Isolation of maytansinoids from plant extracts and separation of maytansinoid mixtures as well as purification of synthetic derivatives usually requires the use of HPLC. A study of the chromatographic behavior of some maytansinoids on a C_{18} reversed-phase and preparative separation on silica gel have been described by Izawa [6]. However, maytansinoids do not dissolve readily in water and are not very stable in aqueous solution. Sample recovery is usually inconvenient from reversed-phase systems.

Since isolation and identification of new maytansinoids is an ongoing project in the laboratory of one of the authors (A.T.S.) a simple normal-phase separation was developed, which can be used for: (1) qualitative analysis of various maytansinoids; (2) screening of crude mixtures for the presence of unknown components; and (3) semipreparative/preparative purification of maytansinoids.

The method should be compatible with a LC–MS

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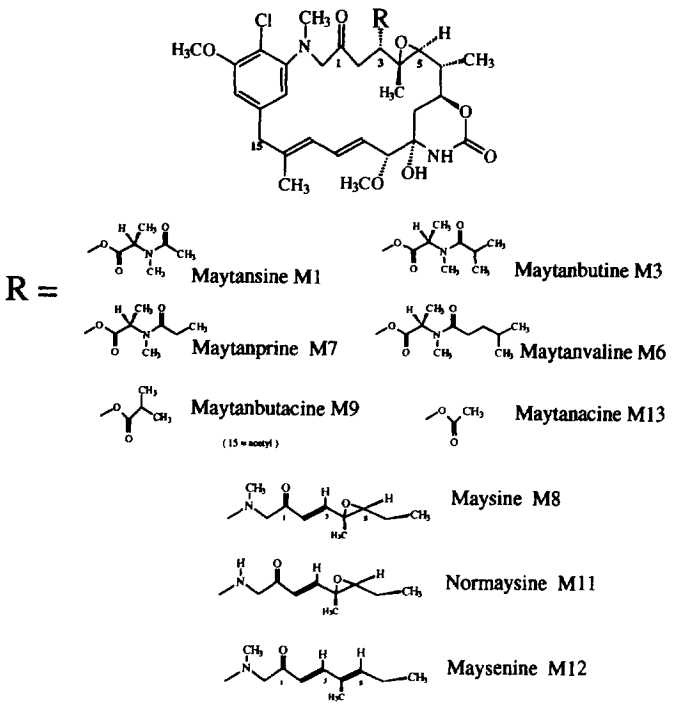

Fig. 1. Structures of maytansinoids.

interface for future studies of unknown compounds. Photodiode array detection was chosen to evaluate the separation and to provide additional information about sample components.

2. Experimental

Samples of maytansinoids were dissolved in dichloromethane prior to injection using a Rheodyne 7125 injector with 10 or 50 μl sample loop. Replicate analysis was performed with a Varian 9300 autosampler (Varian Associates, Walnut Creek, CA, USA). Sample concentration was 1 mg/ml or less, depending on available sample quantities. Chromatography was controlled through a Varian Star Chromatography Workstation ver. 4.5 linked to a Varian 9010 solvent delivery system and a Varian 9065 photodiode array detector. Separations were monitored at 254 nm. All solvents were HPLC-grade or OmniSolv (EM Science, Gibbstown, NJ, USA). Solvents were used without further treatment. The flow-rate of the mobile phase for all separations was set to 1 ml/ml with the exception of 1.5 ml/min for diol-bonded phase.

The following stationary and mobile phases were used:

(A) 150×3.3 mm Separon SGX Si60, 5 μm, HPLC cartridge (silica gel); system A1: isocratic, dichloromethane–methanol (95:5); system A2: isocratic, dichloromethane–dioxane–2-propanol (95:2.5:2.5).

(B) 150×3.3 mm Separon SGX Si60-NH_2, 5 μm, HPLC cartridge (amino-bonded phase); system B1: isocratic, dichloromethane–dioxane–2-propanol (92:4:4); system B2: gradient, dichloromethane–hexane–methanol (80:20:0) to dichloromethane–hexane–methanol (75:20:5) in 5 min.

(C) 150×3.3 mm LiChrosorb CN Si 100, 5 μm, HPLC cartridge (cyano-bonded phase); system C1: isocratic, dichloromethane–hexane–methanol (77:20:3); system C2: gradient, dichloromethane–hexane–methanol (60:40:0) to dichloromethane–hexane–methanol (79:15:6) in 17 min.

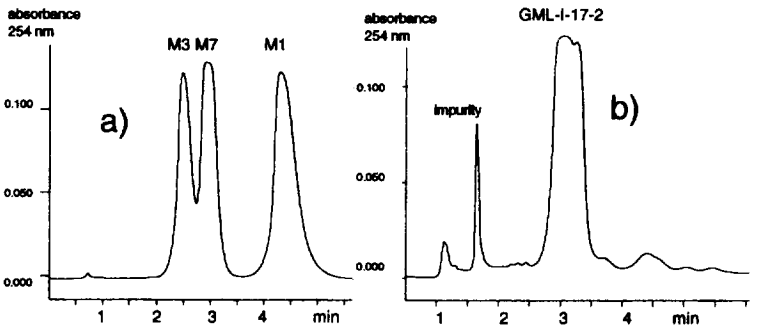

Fig. 2. Separations on silica gel with methanol–dichloromethane as mobile phase. (a) Separation of test mixture; (b) separation of unknown GML-I-17-2.

(D) 250×4.6 mm LiChrosorb diol Si60, 5 μm, stainless steel column (diol-bonded phase); system D1: isocratic, dichloromethane.

Cartridges and column were manufactured by Laboratorny Pristroje, Prague, Cech Republic. Separon SGX is a trademark of Laboratorny Pristroje, Prague. LiChrosorb is a registered trademark of EM Science.

3. Results and discussion

3.1. Semipreparative separations

Based on results of previous work [7] 5% methanol in dichloromethane was evaluated as mobile phase on silica gel. To determine the resolving power of the system a test mixture consisting of the closely related compounds M1, M3 and M7 was used. The compounds are separated but not completely resolved (Fig. 2a). When the same system is used for preparative separation the unknown mixture (GML-I-17-2) yields a major component which is thought to consist of two compounds (Fig. 2b). For comparison the analysis of the test mixture was repeated on a diol-bonded phase using dichloromethane as mobile phase. The separation seems not to be significantly improved (Fig. 3a), however, chromatography of the major component of GML-I-17-2 reveals the existence of 3 components which can be easily separated (Fig. 3b).

It was therefore concluded that a diol-bonded

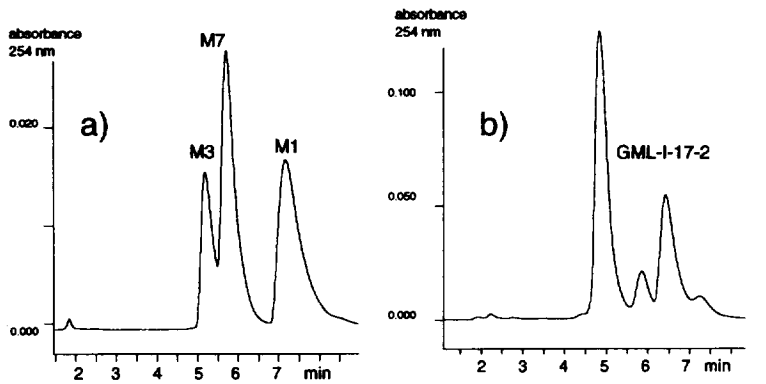

Fig. 3. Separations on diol-bonded phase with dichloromethane as mobile phase. (a) Separation of test mixture; (b) separation of unknown GML-I-17-2.

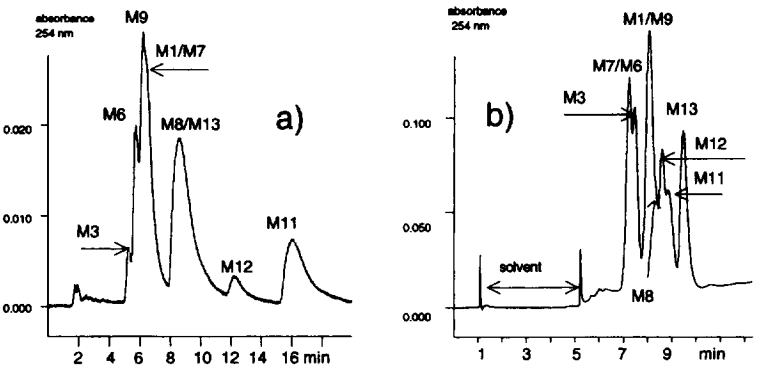

Fig. 4. Separation of nine maytansinoids. (a) diol-bonded phase with dichloromethane as mobile phase; (b) cyano-bonded phase with gradient elution (Section 2).

phase is more suitable for semipreparative separation of maytansinoids than silica gel. Since pure dichloromethane is used as mobile phase sample recovery and mobile phase recycling is more conveniently achieved.

3.2. Investigation of retention of maytansinoids on various stationary phases

A mixture of 9 maytansinoids (M1, M3, M6, M7, M8, M9, M11, M12, M13) was used to optimize separation conditions and to obtain retention data on diol-, aminopropyl- and cyanopropyl-bonded phases as well as on silica gel. Dichloromethane was kept as the main component of the mobile phase. Fig. 4a shows the results on the diol-bonded phase. It is seen that in a complex mixture the most similar compounds M3 and M6 are only partially separated from the co-eluting M1 and M7.

Application of a cyanopropyl-bonded phase required the modification of the mobile phase. One useful mobile phase consisted of 3% methanol in dichloromethane which was diluted with hexane in a ratio of 77:20. Separation of the test mixture was further improved by creating a methanol gradient of zero to 3% methanol with a simultaneous change in the hexane content of the mobile phase from 40 to 30%. It can be seen in Fig. 4b that not all components of the mixture can be separated, however, the order of elution has changed in comparison to the diol-column.

Several attempts have been made to improve separation on either column by changing the selectivity of the solvent. Diethyl ether, 2-propanol, ethyl acetate, as well as acetone were used as polar modifier without significant change in separation performance. High-performance thin-layer chromatography (HPTLC) was therefore employed to systematically optimize the mobile phase for a use on silica gel. A satisfactory separation of the test compounds was achieved with 5% of a dioxane and 2-propanol mixture (1:1) in dichloromethane. On a silica gel column the same mobile phase is very powerful as well (Fig. 5). All pairs of compounds that have created separation problems on other

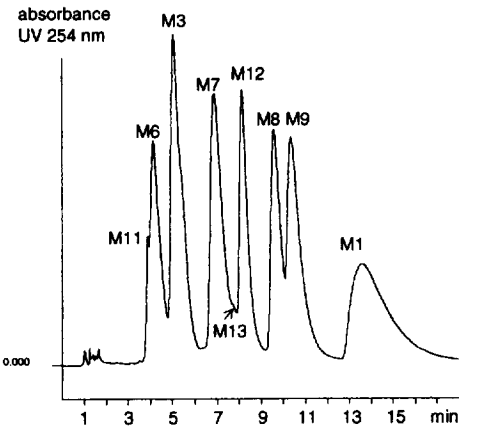

Fig. 5. Separation of nine maytansinoids on silica gel with dichloromethane–dioxane–2-propanol (95:2.5:2.5) as mobile phase.

systems are easily separated. The group M7/M13/M12 as well as the pair M11/M6 can be resolved on the cyanopropyl column (Fig. 4b).

The performance of an amino-bonded phase was evaluated in a gradient method similar to the one used on the cyanopropyl-bonded phase, and with the dioxane-2-propanol modified mobile phase from the silica gel column. Table 1 summarizes the retention times of maytansinoids in different chromatographic systems.

3.3. Spectral analysis of the chromatographic results

With a photodiode array detector UV spectra of all eluting compounds were recorded during separation. Using Polyview software a spectral library was developed from collected raw data. An spectral overlay (Fig. 6) illustrates, that some of the spectra (M8, M12) are significantly different. However, maytansinoids which vary only by one or two carbon atoms in their side chain, give similar spectra. For such spectra optimizing the average wavelength for a given wavelength range weighted by the square of the absorbance, called purity parameter (PuP), allows to distinguish with high certainty between compounds in question. When the PuP for M1, M3, M6 and M7 in the wavelength range of 190 to 239 nm is calculated on silica gel the following values are obtained: M1: PuP 228.865 nm; M3 PuP 227.523 nm; M6 PuP 222.539 nm; M7 PuP 226.059 nm.

Table 1
Retention times (in minutes) of maytansinoids in various chromatographic systems

	A2	D1	C1	C2	B1	B2
M1	13.02	7.78	2.08	7.88	2.19	16.69
M2	4.16	6.30	2.17	7.69	1.75	14.71
M3	5.12	5.62	1.92	7.17	1.45	13.20
M6	4.41	5.14	1.81	6.78	1.48	12.09
M7	7.06	6.17	1.88	6.61	1.87	13.78
M8	9.87	8.02	1.98	8.39	3.58	18.62
M9	10.49	6.13	2.27	7.87	4.49	17.11
M11	3.89	15.07	2.00	9.74	1.28	15.08
M12	8.23	12.03	2.35	8.75	3.15	17.60
M13	7.52	8.36	2.14	9.61	3.41	9.89

See Section 2 for system descriptions.

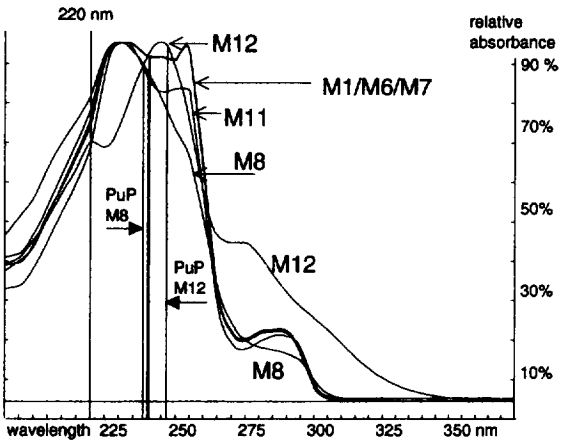

Fig. 6. Overlay of spectra of M1, M6, M7, M8, M11 and M12 after chromatography on silica gel with dichloromethane–dioxane–2-propanol (95:2.5:2.5) as mobile phase.

After examining spectra obtained in different chromatographic systems it became clear, that the UV spectra of maytansinoids are very sensitive to changes in the mobile phase. The PuP of M1 for instance changes in the wavelength range 190 to 239 nm from 223.8 nm in system C1 (see Section 2) to 228.65 nm in system A2 and 219.697 nm in system B2.

Identification of known compounds in the chromatogram is easily accomplished by a comparison of the UV spectrum of the compound in question with a spectral library which was obtained under identical chromatographic conditions. Fig. 7 shows part of the search report documenting the identity of the peak at 8.085 min in Fig. 5 as M12. The match of the unknown spectrum against the library is good even though the peak is not fully resolved.

Multicomponent analysis allows to identify co-eluting compounds, if their spectra are different. Fig. 8 depicts the analysis of the peak pair labeled M7 and M12 in Fig. 5. It reveals, that the peak pair is actually a triplet and compound M13 is co-eluting with M7.

During the investigation of several purified unknown compounds a substance was obtained for which no library match was found. The new maytansinoid M2 is currently investigated in order to detemine its structure.

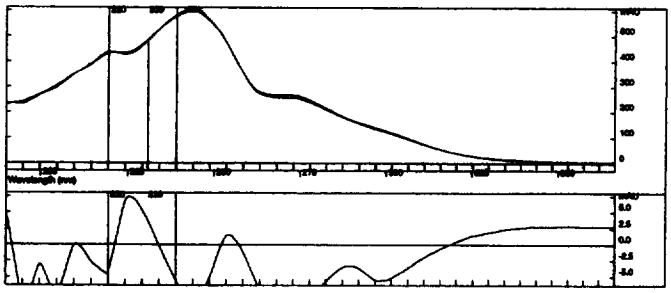

Fig. 7. Part of search report, identifying the peak at retention time 8.085 in Fig. 5 as M12. The upper portion of the picture shows the overlay of the library spectrum (upper curve) and the spectrum of the compound to be identified (lower curve). The lower portion of the picture shows the spectral differences comparing the purity parameter. The match in the wavelength range 220 to 239 nm is 0.99995.

4. Conclusions

Maytansinoids can be separated in the normal-phase mode on diol-, cyanopropyl- and aminopropyl-bonded phases as well as on silica gel.

(1) All investigated stationary phases provide different selectivity for certain compound pairs which makes separation of all known maytansinoids possible.

(2) Orders of elution differ widely from system to system. The best separation of a 9-component test mixture is achieved on silica gel with dioxane–2-propanol in dichloromethane as mobile phase.

(3) The retention mechanism for maytansinoids is not clear. Results suggest a complex interaction of the molecule with the stationary phase based on molecular size and three dimensional structure.

(4) Dichloromethane is a suitable mobile phase for separation. Changes of mobile phase selectivity have only been for in the case of dioxane, which yields excellent results as polar modifier.

(5) Spectral libraries for the various chromatographic systems are helpful for the determination of known compounds and analysis of purity.

(6) With the developed methods unknown mixtures from plant extracts and synthetic work can be easily screened for the presence or absence of known maytansinoids.

A previously unknown maytansinoid M2 was purified. The structure of the compound is currently under investigation.

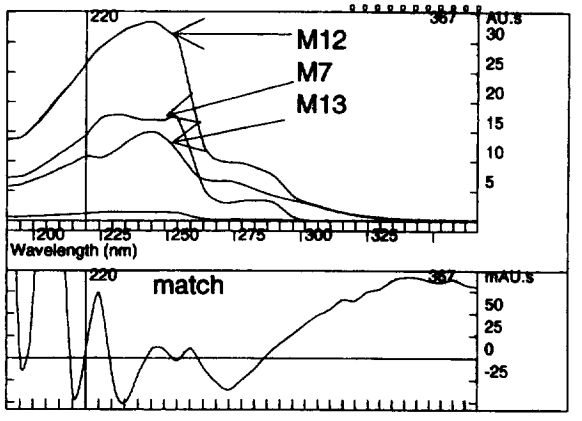

Fig. 8. Part of a multicomponent analysis identifying the shoulder between peaks M7 and M12 in Fig. 5 as M13.

Acknowledgments

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