

Purine and Related Compound

Purification Strategies

Chromatography Application Note AN59

Abstract

Purines and their derivatives are a common ring system found in medicinal chemistry and natural products. As these compounds are so common, methods to purify them are of interest to chemists. This application note lists compounds from the literature and the methods used to purify them.

Introduction

Purines are the most common nitrogen-containing heterocycle found in nature¹. Purines are found in nucleotides and are common in natural products as xanthine alkaloids.

Purines are purified as other small molecules. Despite the multiple nitrogens in the ring systems, purines and related compounds are easily purified. The compound polarity is modulated by substituents on the ring system. In general, non-polar substituents tend to allow silica purification with hexane/ethyl acetate. Purines with few substituents, or containing polar substituents, are generally purified using dichloromethane/methanol or under C18 reversed phase conditions. Amine columns are also useful to purify this class of compounds.

Purification Strategies

Silica Gel

The first examples are relatively non-polar purine compounds. These tend to have substituents such as benzyl attached that reduce the overall polarity of the compound. These are purified with hexane/ethyl acetate solvent systems although one example, Compound 2, uses ethyl acetate with some methanol. The presence of the amine and hydroxyl groups increases the polarity of Compound 2 sufficiently as to require 1% methanol.

Compound 7 is interesting because it is purified with ethyl acetate and methanol with a basic modifier. Ethyl acetate might be used in this purification because it is more polar than dichloromethane². Alternatively, since the polarity could have been increased by increasing the concentration of methanol, ethyl acetate was employed because it provided differing selectivity of the target compound from impurities as compared to dichloromethane.

Despite the basic nature of the purine ring system, few purifications required modifiers such as triethylamine. Compound 7 is an exception.

In general, silica gel is widely used because the columns are inexpensive. Typically, there is little need for solvent modifiers allowing for easier purifications. The solvents have low boiling points and are easily removed.

Table 1: Purification of Non-polar Purines on Silica Gel Columns

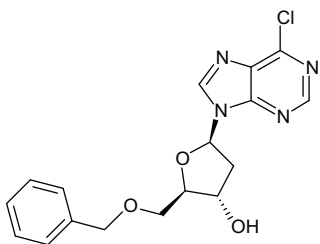
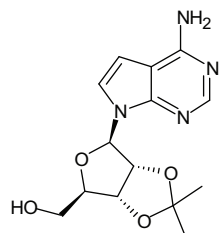
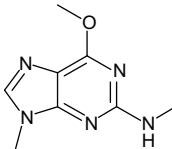
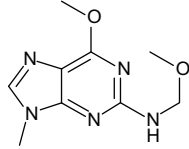
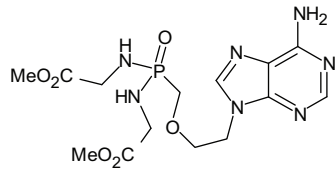
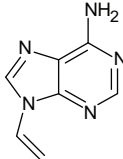
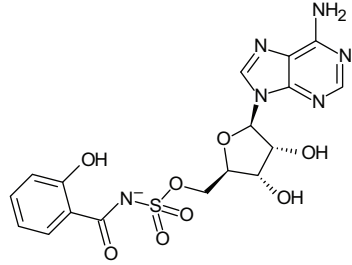
ID and Reference	Structure	Name	Solvent System
1 ³		9-(5-O-benzoyl-b-D-2-deoxyribofuranosyl)-6-chloropurine	ethyl acetate: hexane gradient, 2:1 to 3:1
2 ⁴		7-Deaza-2',3'-O-isopropylideneadenosine	ethyl acetate: methanol, 99:1

Table 2: Purification of Moderately-polar Purines and Related Compounds

ID and Reference	Structure	Name	Solvent System
3 ⁵		6-Methoxy-N,9-dimethyl-9H-purin-2-amine	0–5% methanol in dichloromethane
4 ⁶		6-Methoxy-N-(methoxymethyl)-9-methyl-9H-purin-2-amine	2–10% methanol in dichloromethane
5 ⁷		9-(Bis(N,N'-(methoxycarbonylmethyl)phosphonamido)methoxyethyl)adenine	1–10% methanol in dichloromethane
6 ⁸		9-vinyladenine	chloroform: methanol, 9:1
7 ⁹		7-Deaza-5'-O-[N-(2-hydroxybenzoyl)sulfamoyl]adenosine Triethylammonium Salt	ethyl acetate: methanol: triethylamine, 90:10:1

C18 Purification Strategies

Table 3 shows that compounds containing purine rings can be purified with C18 columns. When running reversed phase solvent systems, it is often necessary to add trifluoroacetic acid (TFA), formic acid, or another modifier to keep the peaks sharp. For flash chromatography, the modifier should be a compound that is easily

removed. Formic acid, acetic acid, TFA, and ammonium acetate are removed during lyophilization.

Acid labile compounds can be run on RediSep Gold C18 under basic conditions (Figure 1). Ammonia was used as the additive because it can be removed during lyophilization. The end-capping on the RediSep Gold C18 provides some protection from basic conditions.

Table 3: Purification of Purines and Related Compounds on C18 Columns

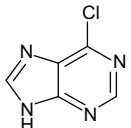
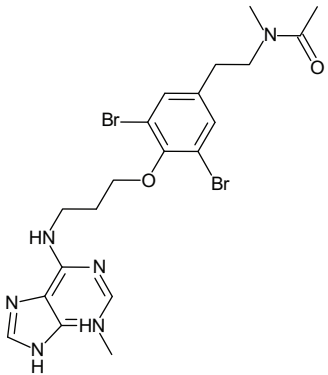
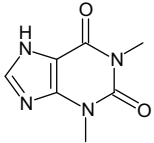
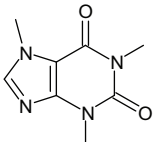
ID and Reference	Structure	Name	Solvent System
8 ¹⁰		6-chloropurine	methanol: water, 1:1

Table 3: Purification of Purines and Related Compounds on C18 Columns (Continued)

ID and Reference	Structure	Name	Solvent System
9 11		aphrocallistin	water: acetonitrile gradient, 0.1% TFA
10 12		theophylline	water (pH 10, ammonia): methanol, RediSep Rf Gold C18
11 12		caffeine	water (pH 10, ammonia): methanol, RediSep Rf Gold C18

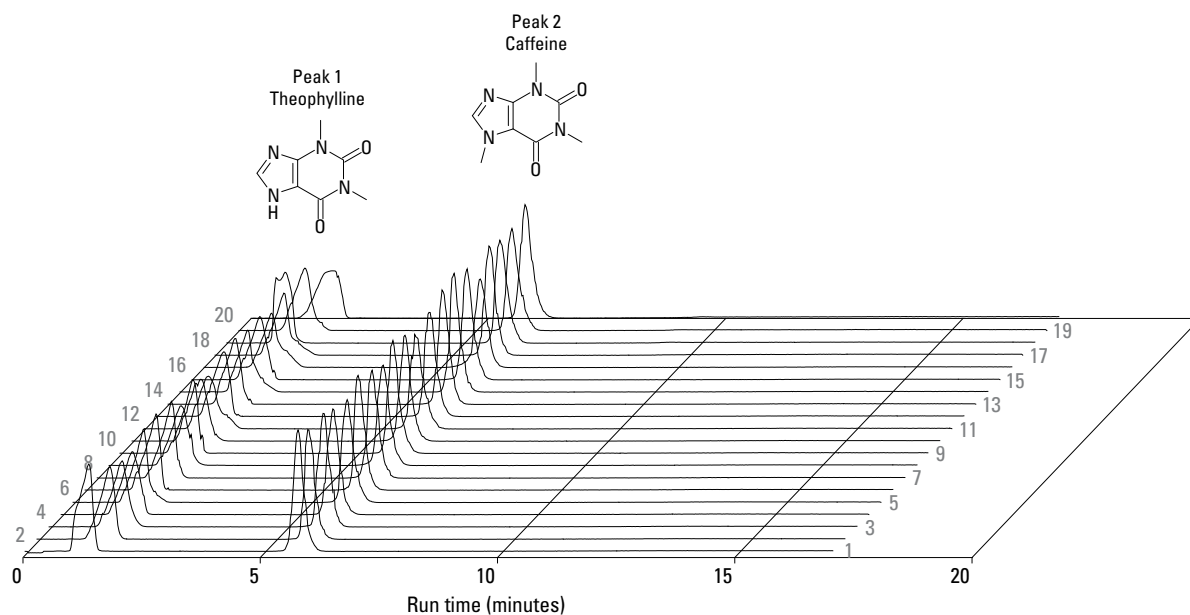


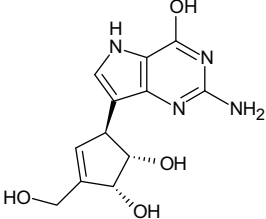
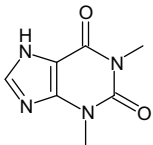
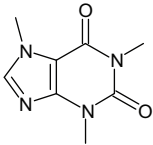
Figure 1: Repeated injections of caffeine and theophylline on RediSep Rf Gold C18 under basic conditions

Amine Purification Strategies

Amine functionalized silica provides another purification method for purine compounds. This bonded phase is generally run as a normal phase material with the same solvents that are used for silica. Since the amine bonded phase is less polar than the surface of

un-modified silica, compounds retain less strongly on the amine bonded phase compared to silica. The basic nature of the bonded phase means that solvent modifiers may not be required.

Table 4: Purification of Non-polar Purines on Amine Columns

ID and Reference	Structure	Name	Solvent System
12 13		(1'S,2'S,3'R)-9-(2',3'-Dihydroxy-4'-hydroxymethyl-4'-cyclopenten-1'-yl)-deazaguanosine	dichloromethane: methanol, 5:1
10		theophylline	acetonitrile: water gradient
11		caffeine	acetonitrile: water gradient

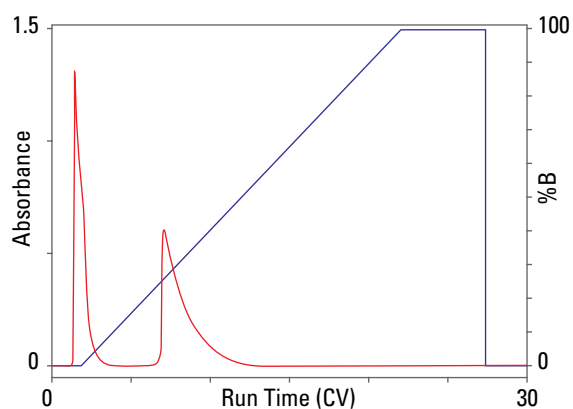


Figure 2: Amine Column with Aqueous Normal Phase Conditions to purify theophylline and caffeine

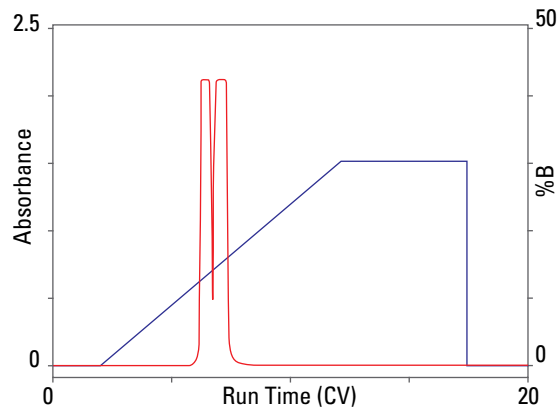


Figure 3: Silica Column with Dichloromethane/Methanol Gradient to purify theophylline and caffeine

Despite the large number of hydroxyl groups and an amine group that make Compound 12 very polar, it was eluted from the amine column with dichloromethane and methanol.

The amine column may provide differing selectivity to compounds compared to silica, allowing resolution of compounds that may otherwise be difficult to purify. Compounds 10 and 11 were run on an amine column using aqueous normal phase. Much better resolution between the peaks was noted compared to a run on silica using a dichloromethane: methanol gradient. This experiment also demonstrates the possibility that purine derivatives can be resolved without the use of chlorinated solvents.

Conclusion

Purine compounds are easily purified on flash chromatography systems. Modern systems, such as the CombiFlash Rf-200, allow use of a variety of column types and solvent systems to allow the best purification of purines and related compounds.

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