JOURNAL OF FORENSIC SCIENCES

*J Forensic Sci,* March 2010, Vol. 55, No. 2
doi: 10.1111/j.1556-4029.2009.01267.x
Available online at: interscience.wiley.com

**TECHNICAL NOTE**
**CRIMINALISTICS**

*Sandra E. Rodriguez-Cruz,*[1] *Ph.D. and Kathryn A. Carson,*[1] *B.S.*

# Anion Identification via Complexation with *meso*-octamethylcalix(4)pyrrole and Detection Using Electrospray Ionization Mass Spectrometry*

**ABSTRACT:** The routine identification of controlled substances and adulterants during forensic chemistry analysis often involves the identification of counter ions or salt forms present in an exhibit. Here, the use of the compound *meso*-octamethylcalix(4)pyrrole (C4P) during salt-form identification analysis is presented. C4P is a commercially-available, anion-binding agent that can be reacted with a controlled substance or adulterant, resulting in the sequestration of anionic species, usually present as counter ions to the active ingredient. Formation of noncovalent complexes between the cyclic host C4P compound and anionic guests is investigated using electrospray ionization–mass spectrometry (ESI–MS). Complexes with chloride, bromide, iodide, nitrate, and acetate are readily observed and mass spectrometry analysis provides identification via molecular weight characterization. Chloride and bromide complexes are also characterized by the isotopic distribution of their molecular ions. Formation of host–guest complexes is not observed for sulfate and phosphate salts, presumably due to steric hindrance and energetically unfavorable conditions.

**KEYWORDS:** forensic science, forensic chemistry, drug analysis, anion identification, *meso*-octamethylcalix(4)pyrrole, electrospray ionization–mass spectrometry, noncovalent complex

Most basic controlled substances, as commonly encountered on the street, can exist as amorphous compounds, fine powders, or as crystalline solids. These can be described with the general chemical formula M•HX, where the controlled substance M exists in a protonated state $MH^+$ accompanied by a negatively charged species $X^-$ like chloride ($Cl^-$), bromide ($Br^-$), or nitrate ($NO_3^-$), among others. Chemically, the controlled substance is then said to be found in a salt form, as opposed to its base form. In addition to the routine identification of controlled substances and adulterants present in an exhibit, forensic chemistry analysis often also involves the identification of these counter ions or salt forms. Compared to the free form of a controlled substance, salt formation often results in modifications of the physical and chemical properties of the molecule. Furthermore, identification of the salt form of a controlled substance becomes an essential part of the forensic analysis protocol when the results lead to significantly different sentencing penalties under current U.S. Federal law, like in cases involving cocaine base and cocaine HCl (1). Salt-form determination can also provide evidence that an outwardly licit pharmaceutical preparation is counterfeit, if the identified salt form is not the form known to be used by the legitimate manufacturer (2).

For many years, salt-form or anion identification in forensic chemistry laboratories has involved the use of numerous presumptive tests (3–5). The most common ones involve the use of aqueous solutions of silver or barium nitrate and allow indirect identification of the unknown anion by formation and precipitation of a water-insoluble salt (4,5). The silver nitrate ($AgNO_3$) test is routinely used during the presumptive determination of halides. During this test, an aqueous solution of $AgNO_3$ is added to an aqueous solution of the unknown salt. The presence and color of a precipitate are indicative of the type of anion present. For example, upon reaction with $AgNO_3$, the anions $Cl^-$ and $Br^-$ form white (AgCl) and pale yellow (AgBr) precipitates, respectively. On the other hand, determination of some nonhalide anions like sulfates ($SO_4^{2-}$), phosphates ($PO_4^{3-}$), and nitrates ($NO_3^-$) is better accomplished using the barium nitrate $Ba(NO_3)_2$ test (4,5), as these anions do not form precipitates with silver.

Infrared (IR) spectroscopy is another technique often used for salt-form identification during the analysis of controlled substances. The IR spectral region between 2000 and 3500 $cm^{-1}$ offers distinctive absorption bands that allow the differentiation of multiple salt forms. However, analysis by IR requires high purity samples, or otherwise the use of dry extractions and recrystallization techniques to obtain a clean product. These processes can also lead to different crystalline forms or polymorphs, making conclusive identification of the salt form difficult and often impossible. IR has also been used for the indirect determination of anions in aqueous acids by reaction with a known basic compound and production of the respective salt form (6).

Inorganic anions have also been analyzed by ion chromatography (IC) (7), capillary electrophoresis (CE) (8,9), and gas chromatography/mass spectrometry (GC–MS) (10,11). IC and CE are often considered complementary techniques that allow for the detection of multiple anionic species during the same experiment. However,

[1]U.S. Drug Enforcement Administration, Southwest Laboratory, 2815 Scott Street, Vista, CA 92081.

*Presented at the American Academy of Forensic Sciences Annual Meeting, February 16–21, 2009, in Denver, CO.

Received 23 Oct. 2008; and in revised form 28 Jan. 2009; accepted 31 Jan. 2009.

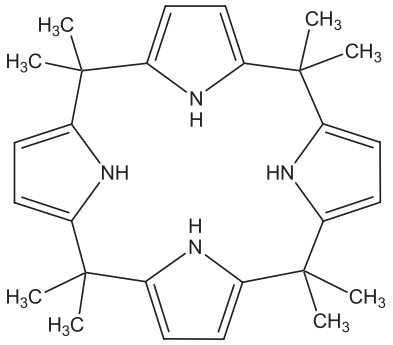


FIG. 1—*Structure of* |*bital*|*meso*|*eital*|*-octamethylcalix(4)pyrrole (C4P).*

extensive preparation steps and relatively long analysis times limit their applicability during routine operations. GC–MS methods combine anion separation with selective and sensitive detection. However, the low volatility of inorganic anions requires the use of time-consuming derivatization techniques.

The compound *meso*-octamethylcalix(4)pyrrole (C4P; Fig. 1) is one of the most versatile anion binding receptors in solution. Originally synthesized more than 120 years ago by Baeyer (12), C4P is commercially available or can be easily produced via the condensation of pyrrole and acetone. During the last 10 years, this type of compound has formed the basis for the production of an extensive series of anion-binding compounds. Solid and solution-state studies by Sessler et al. have demonstrated that the C4P and analogous systems are effective 1:1 anion-binding agents (13,14). During these studies, a high degree of selectivity has also been demonstrated, where the C4P system shows a binding preference for $F^-$ relative to other investigated anions $Cl^-$, $Br^-$, $I^-$, $H_2PO_4^-$, and $HSO_4^-$. X-ray crystallography analyses of the complexes between fluoride, chloride, and C4P indicate the anions are located above the plane formed by the four pyrrole nitrogens (13,14). In solution, variable temperature $^1H$ NMR studies suggest that C4P adopts a cone conformation when bound to the $F^-$ anion. Solution and solid-state studies demonstrate that the calixpyrrole systems also show a decreased but evident affinity to neutral species. During these interactions, however, coordination appears to be dominated by steric effects (14). The

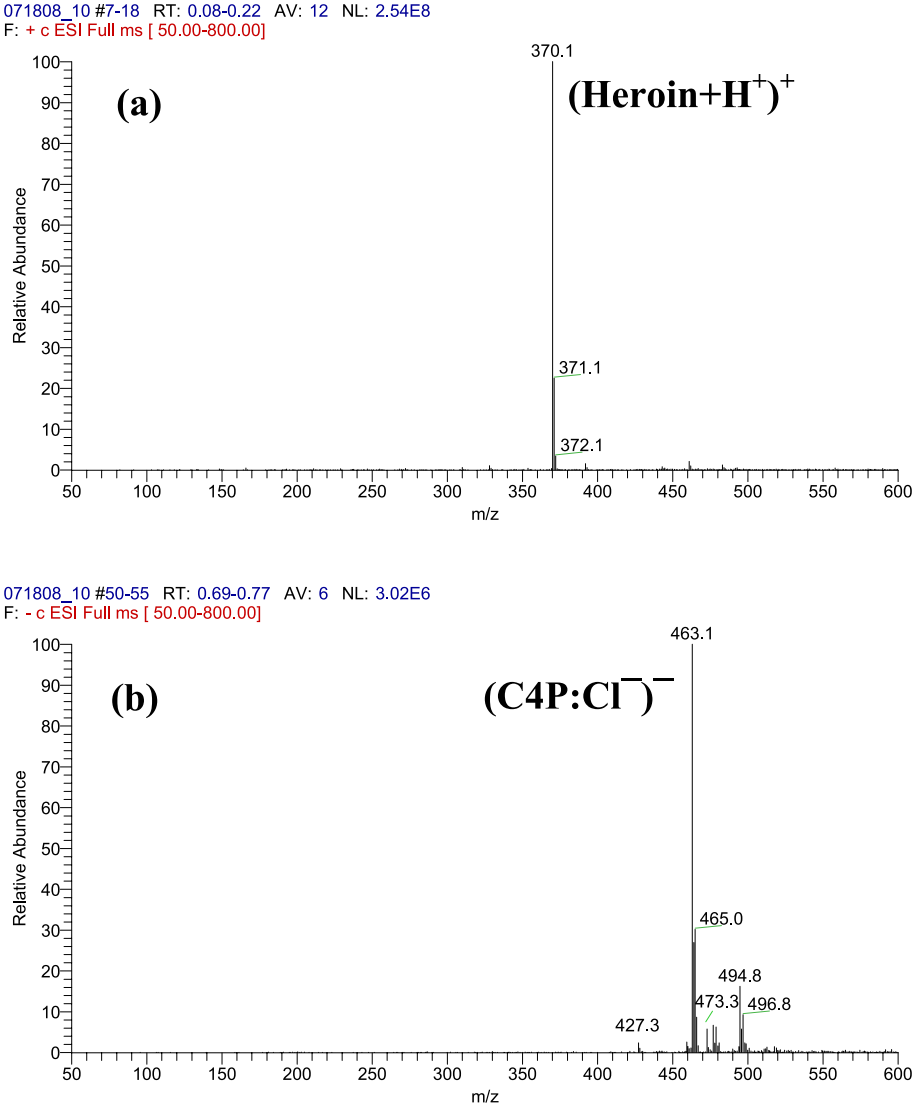


FIG. 2—*Positive (a) and negative (b) electrospray ionization mass spectra of a solution containing heroin HCl and* |*bital*|*meso*|*eital*|*-octamethylcalix(4)pyrrole.*

synthesis of larger calix(*n*)pyrrole ($n$ = 5, 6) systems has also been reported in the literature (15,16). However, contrary to the C4P system, the larger cyclooligomers are not commercially available, as they can only be produced in relatively low yields.

The anion-binding C4P system can be used for the rapid identification of some of the most common salt forms encountered in the forensic chemistry laboratory. The simple addition of C4P to a solution of a controlled substance (or adulterant) salt results in the sequestration of the anionic species by the C4P cyclic compound. The noncovalent complex formed is negatively charged with a gas-phase stability proportional to the affinity between the guest (anion) and the host (C4P) molecule. Analysis via electrospray ionization (ESI) (17,18) provides the ideal process for transporting these noncovalent complexes from solution into the gas phase, where they can be conclusively identified in a simple mass spectrometry experiment. This paper will present the application of this methodology to the rapid identification of multiple anions routinely encountered in the forensic chemistry laboratory.

## Experimental

Experiments were performed using a Thermo Fisher Scientific (San Jose, CA) LCQ Advantage MAX quadrupole ion-trap mass spectrometer equipped with an IonMAX atmospheric pressure ionization source and an electrospray ionization probe. Cocaine HCl and dextromethorphan (DXM) HBr were obtained from Sigma Chemical Co. (St. Louis, MO). Heroin HCl was obtained from the authenticated standard collection of the DEA Special Testing and Research Laboratory (Dulles, VA). The iodide, nitrate, and acetate salts of ephedrine were synthesized in-house from commercial ephedrine HCl (Sigma Chemical Co.) and are part of the authenticated standard collection of the DEA Southwest Laboratory. Test solutions were prepared at concentrations of 10–15 μg/mL using acetonitrile (ACN) as the solvent. A stock solution of *meso*-octamethylcalix(4)pyrrole (C4P) (97% purity, Sigma-Aldrich, Milwaukee, WI) was prepared at a concentration 0.5 mg/mL using acetonitrile. In order to induce complex formation, 50 μL of the C4P stock solution was added to 1 mL of each test solution. All solutions were prepared using acetonitrile as the solvent, since the use of any protic solvents significantly interferes with the formation of the noncovalent complexes between the C4P and the anions. Prior to the experiments, the syringe and electrospray solvent lines were also thoroughly rinsed using acetonitrile. All tests solutions were freshly prepared and analyzed before and after addition of the pyrrole stock solution. Solutions were injected into the mass spectrometer using the built-in syringe pump and infused at a flow rate of 5 μL per minute.

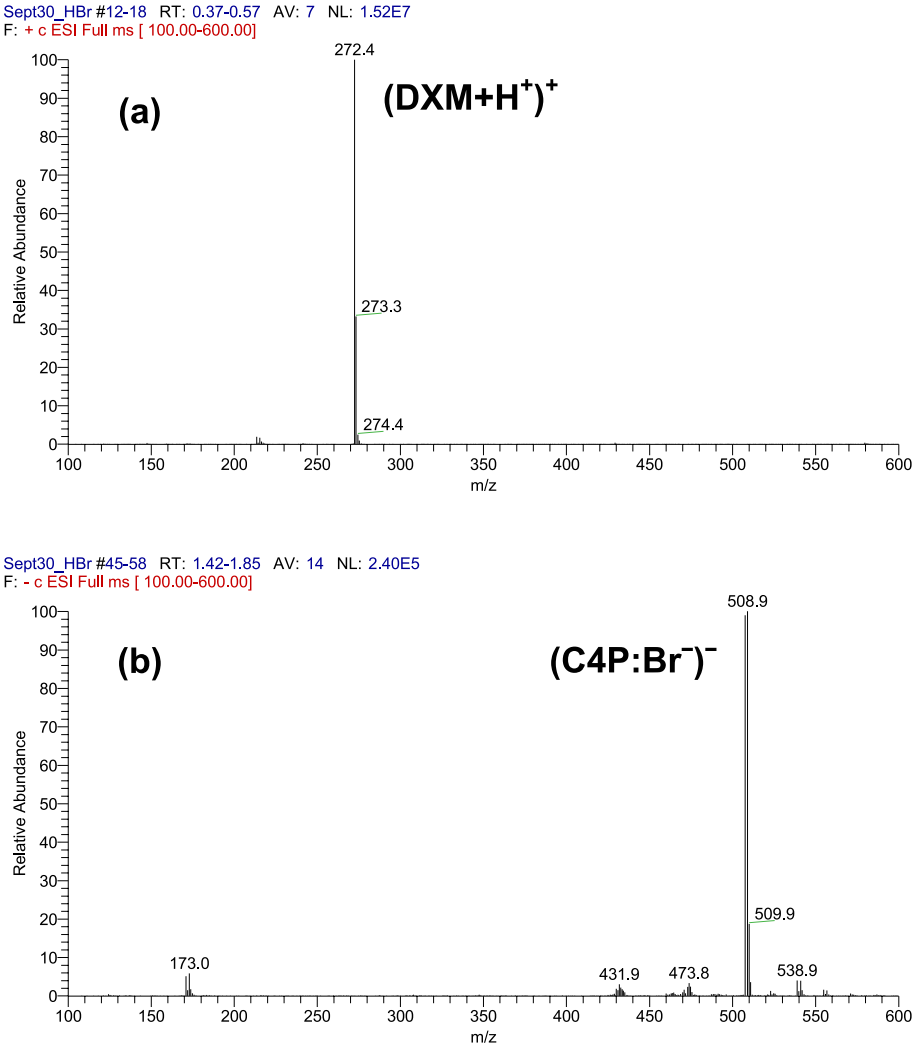


FIG. 3—*Positive (a) and negative (b) electrospray ionization mass spectra of a solution containing dextromethorphan (DXM) HBr and *bital*\|meso\|*eital*\|-octamethylcalix(4)pyrrole.*

For the ESI–MS experiments, the electrospray voltage was operated at ±4.0 kV and the source transfer capillary was maintained at a temperature of 200°C and a voltage of ±11 to 20 V. The tube lens voltage was manually varied between 10 and 30 V, depending on the salt solution analyzed. The electrospray desolvation process was aided using Nitrogen (99%; 100 ± 20 psi) as sheath and auxiliary gas, both operated at 5 units. Ions were injected into the quadrupole ion trap with a maximum injection time of 300 milliseconds. Helium (99.999%; 40 ± 10 psi) was used as the trapping gas. Mass spectrometry data were collected in both positive and negative ion modes using the real-time view provided by the *Tune Plus* program module, and ions were analyzed by collecting full-scan mass spectra between 50 and 600 mass-to-charge (m/*z*) units. Once detection of the protonated molecular ion $(M+H^+)^+$ has been recorded in positive ion mode, simple automatic switching of the analyzer's polarity allowed detection of the anion complex $(C4P:X^-)^-$. Data collection was completed in less than 2 min. Instrument control, data collection, and analysis were performed using the *Xcalibur*™ software (version 1.4) provided by the instrument manufacturer.

## Results and Discussion

### *Halides*

Figure 2 shows the electrospray ionization mass spectra obtained during analysis of a solution containing *c.* 10 μg/mL of heroin (MW = 369 Da) HCl and 14 μg/mL of *meso*-octamethylcalix(4)pyrrole (MW = 428 Da). The positive ion mode spectrum (Fig. 2*a*) is dominated by the protonated heroin signal at m/*z* 370. The negative ion mode spectrum (Fig. 2*b*) shows the presence of m/*z* 463, corresponding to the negatively charged complex between *meso*-octamethylcalix(4)pyrrole and the chloride anion $(C4P:Cl^-)^-$. The rapid identification of the anion is achieved via confirmation of the molecular weight of the complex (428 + 35 = 463) and by observation of the isotopic distribution of the molecular ion. A close look at such signal confirms the presence of the A and A + 2 ions at m/*z* 463 and 465, respectively, with the latter ion showing an intensity characteristic of the isotopic abundance of $^{37}Cl$ (32%). Also observed in the negative ion mode spectrum is a signal at m/z 495. Secondary signals 32 mass units above the anion complex are due to methanol binding and are discussed later in the manuscript.

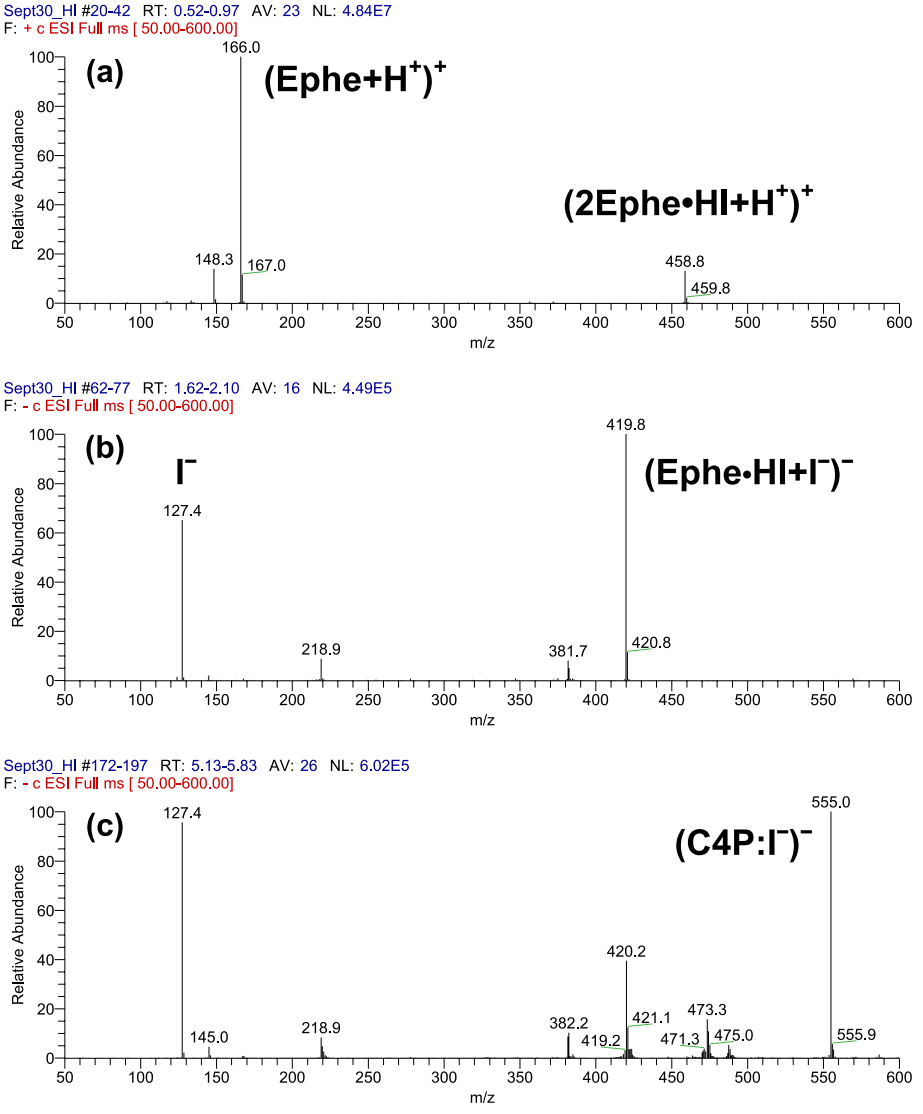


FIG. 4—*Electrospray ionization spectra of a solution containing ephedrine HI before (a and b) and after (c) addition of |*bital*|meso|*eital*|-octamethylcalix(4)pyrrole.*

Rapid anion identification is also achieved during analysis of a solution containing dextromethorphan (DXM; MW = 271 Da) HBr and C4P. Figure 3 shows positive (a) and negative (b) ESI–MS data confirming detection of the protonated dextromethorphan at m/*z* 272 and the $(C4P:Br^-)^-$ complex at m/*z* 507, respectively. As in the case of chloride, the negatively charged complex with bromide dominates the negative ESI–MS spectrum and its isotopic distribution is consistent with that expected for a compound containing one bromide anion.

Figure 4 shows results for the analysis of a solution containing ephedrine hydroiodide (HI) before and after addition of C4P. The positive ESI spectrum (Fig. 4*a*) shows a dominating protonated molecular ion at m/*z* 166 and an ephedrine fragment at m/*z* 148. Also observed is an ion at m/*z* 459 due to the proton-bound dimer of ephedrine and ephedrine•HI. Before addition of the C4P compound, the negative ESI spectrum (Fig. 4*b*) indicates ions at m/*z* 127 and 420. These correspond to iodide and $(ephedrine•HI + I^-)^-$, respectively. After addition of C4P, the formation of the complex $(C4P:I^-)^-$ at m/*z* 555 is observed (Fig. 4*c*). However, contrary to the experiments with $Cl^-$ and $Br^-$, the iodide complex is not the only dominant product. Signals for $I^-$ (m/*z* 127) and ephedrine•HI + $I^-$ (m/*z* 420) are still observed, in addition to a lower intensity signal at m/*z* 473.

Results obtained during the anion binding of $Cl^-$, $Br^-$, and $I^-$ by *meso*-octamethylcalix(4)pyrrole indicate an affinity trend that mimics that previously observed by Sessler et al. in solid and solution-state binding studies (13,14). That is, C4P demonstrates a binding preference for the smaller ions as indicated by the dominance of the $(C4P:Cl^-)^-$ complex relative to the $(C4P:Br^-)^-$ and $(C4P:I^-)^-$ complexes. For the larger anions, the complexation process competes with the binding of neutrals and/or other smaller size ions, even though these latter species are probably present at significantly lower concentrations.

The expected differences in solution and gas-phase affinity between different anions can be best illustrated by investigating the competitive binding of C4P with two different ions. Figure 5 shows the results of such a competition experiment after the addition of C4P to a solution containing equimolar amounts of cocaine HCl and dextromethorphan HBr. The positive ESI spectrum (Fig. 5*a*) shows protonated molecular ions at m/*z* 272 and 304 corresponding to dextromethorphan and cocaine, respectively. The negative ESI spectrum (Fig. 5*b*), however, is dominated by $(C4P:Cl^-)^-$ at

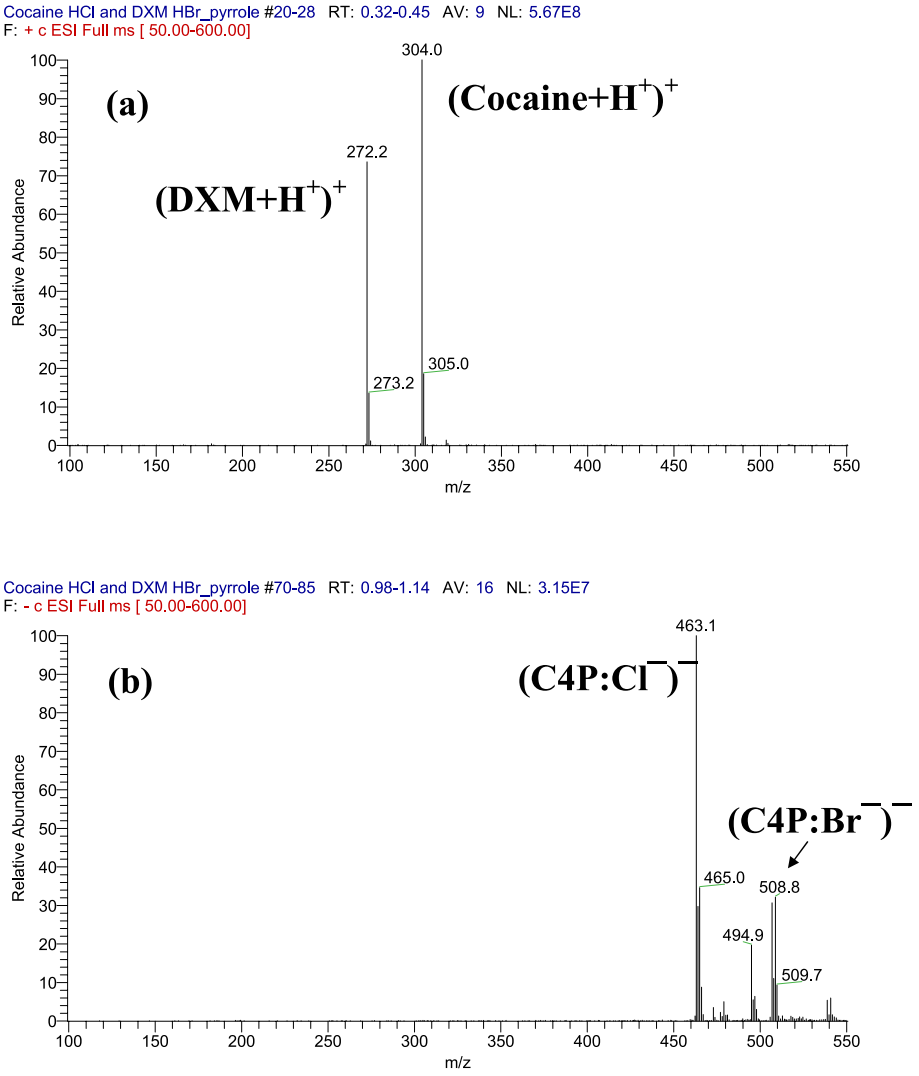


FIG. 5—*Positive (a) and negative (b) electrospray ionization mass spectra of a solution containing |*bital*|meso|*eital*|-octamethylcalix(4)pyrrole and equimolar amounts of cocaine HCl and dextromethorphan (DXM) HBr.*

m/z 463. The formation of $(C4P:Br^-)^-$ at m/z 507 is also observed, but this complex is only present with an abundance of *c.* 35% relative to that of the chloride complex. This result reflects the higher binding affinity of C4P to the smaller anion, and is consistent with previous solution and solid-state reports (13,14).

Most of the negative electrospray ionization spectra collected for the different salts also show the presence of a low intensity signal at m/z 473. MS/MS and $MS^3$ fragmentation studies of this peak (results not included) indicate this ion is due to formation of a complex between C4P and formate ($HCO_2^-$). The latter compound is expected to be present at low levels during these experiments because formic acid is an additive routinely used in this instrument. As mentioned during the experimental section, thorough rinsing of the electrospray source and solvent lines using acetonitrile is highly recommended before anion analysis using C4P. Also, the unintentional addition of protic solvents or other interfering anions favors the formation of deprotonated C4P (m/z 427) and the formate complex, while decreasing the abundance of the $(C4P:X^-)^-$ complexes (see also Fig. 8 and discussion below). Isolation of the electrospray ionization chamber is also recommended in order to reduce interference and complexation of neutrals present in the laboratory atmosphere.

### *Nitrates and Acetates*

Anion binding by *meso*-octamethylcalix(4)pyrrole can also be used for identification of other nonhalide anions present in a sample. Figures 6 and 7 demonstrate the use of C4P during the analysis of nitrate ($NO_3^-$) and acetate ($CH_3CO_2^-$) salts, respectively. Addition of C4P to solutions of ephedrine $HNO_3$ and ephedrine acetate results in the formation of $(C4P:NO_3^-)^-$ and $(C4P:CH_3CO_2^-)^-$. These complexes are detected at m/z 490 and 487, respectively.

### *Methanol Binding*

Under the current experimental conditions, the appearance of secondary signals 32 mass units above the expected $(C4P:X^-)^-$ complexes is commonly observed during the analyses of chloride, bromide, and nitrate salts (Figs. 2, 3, 5, and 6). These signals can be detected at m/z values of 495, 539, and 522, respectively. These ions are probably due to the binding of one neutral methanol molecule to each one of the complexes and would be consistent with previous findings by Sessler et al. (14) indicating that the C4P system can also effectively interact with small neutral species. These results are not unexpected, because methanol is a ubiquitous and

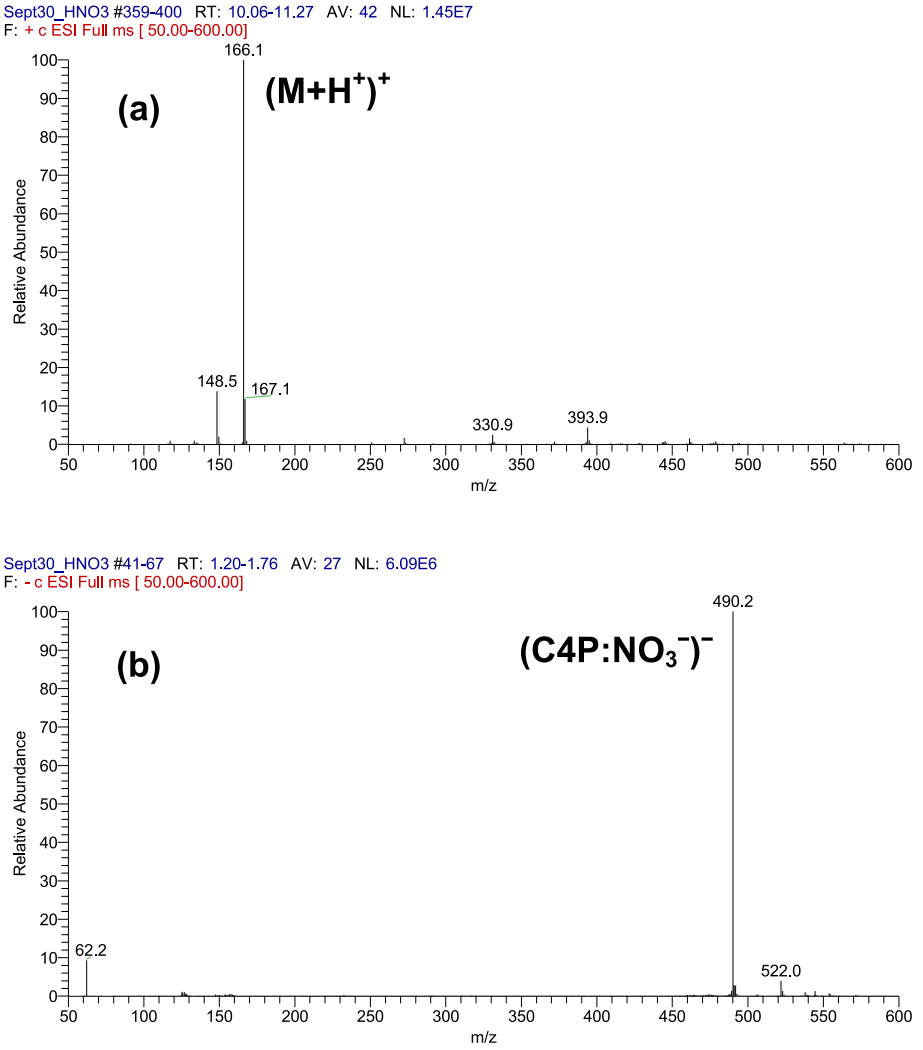


FIG. 6—*Positive (a) and negative (b) electrospray ionization mass spectra of a solution containing ephedrine HNO|*bsub*|3|*esub*| and |*bital*|meso|*eital*|-octamethylcalix(4)pyrrole.*

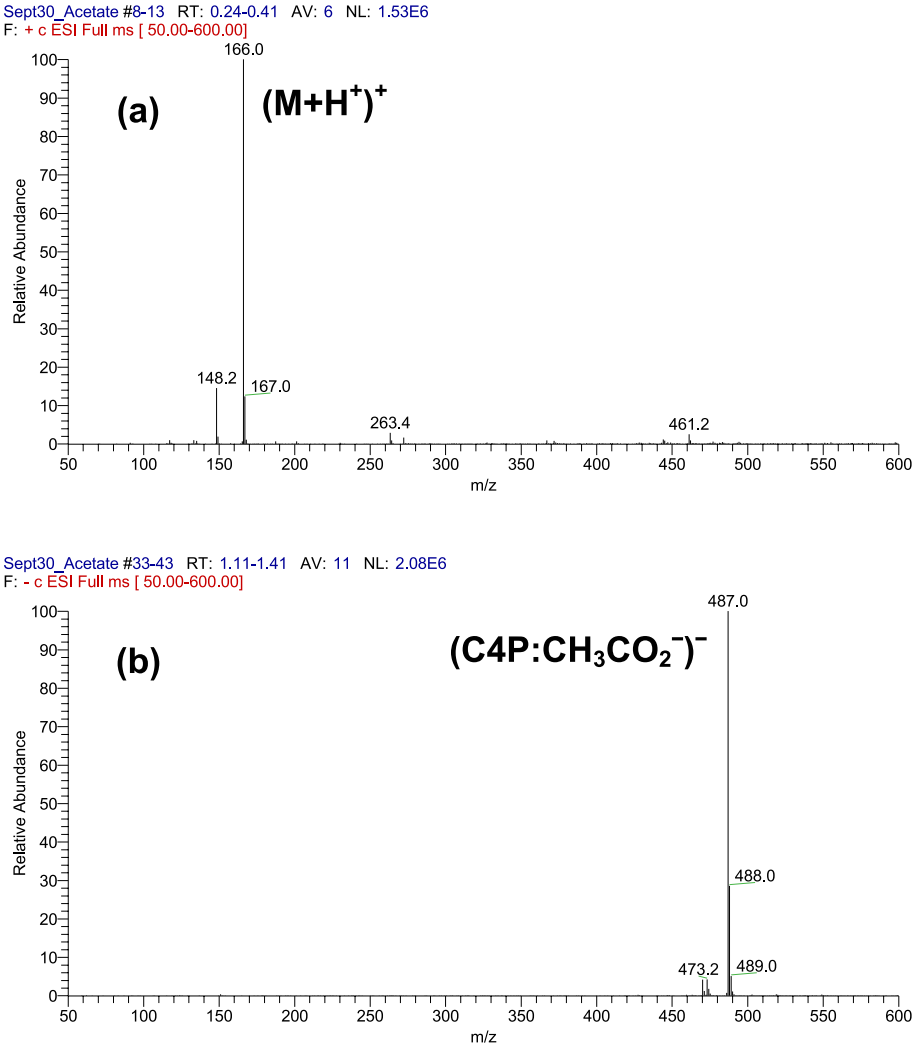


FIG. 7—*Positive (a) and negative (b) electrospray ionization mass spectra of a solution containing ephedrine acetate and* |*bital*|*meso*|*eital*|*-octamethylcalix(4)pyrrole.*

routinely used solvent in this instrument. The interference of methanol can be eliminated if instrument use is periodically restricted to anion-binding experiments. Otherwise, thorough rinsing of the solvent lines is necessary to significantly reduce the detection of methanol adducts.

### *Sulfates and Phosphates*

Addition of *meso*-octamethylcalix(4)pyrrole to solutions containing sulfate ($SO_4^{2-}$) and phosphate ($PO_4^{3-}$) salts did not produce the expected complexes. Previous studies by Sessler et al. (13,14) reported the formation of complexes between the C4P system and the singly charged anions dihydrogen phosphate ($H_2PO_4^-$) and hydrogen sulfate ($HSO_4^-$). Their solution measurements indicate the stability constant for the $(C4P{:}H_2PO_4^-)^-$ complex is intermediate between those measured for the $(C4P{:}Cl^-)^-$ and $(C4P{:}Br^-)^-$ complexes, while the measured stability constant for the $(C4P{:}HSO_4^-)^-$ system is comparable to that of $(C4P{:}I^-)^-$. The absence of sulfate and phosphate complexes in our studies suggests that formation of the host–guest systems is not occurring for the doubly and triply charged ions, and is probably a direct result of the higher desolvation energies necessary prior to the sequestration by the C4P system.

Although to a lesser extent, the size and geometry of the sulfate and phosphate ions might also be important factors during the formation of the C4P complexes. The four pyrrole NH moieties in the center of the C4P system are expected to interact more favorably, via hydrogen bonds, with spherical charges like the halide anions or with planar geometries like those of nitrate, acetate, and formate. The larger size and tetrahedral geometry of the sulfate and phosphate anions result in a less than ideal environment for the host–guest interactions to occur. The absence of complexes between C4P and these anions suggests that the most favorable interactions with the pyrrole NH groups have been disrupted.

### *Salt Form Identification*

The methodology presented here is a valuable tool during analysis and identification of the salt form of a controlled substance or adulterant. However, anion identification should not be solely based on the formation of these complexes. If possible, supplementary tests like IR and/or precipitation tests should also be utilized during analysis. The use of C4P as anion-binding and identification agent is more appropriate in cases where only one salt form is suspected or when the salt form is present at nontrace levels. For salt mixtures, C4P complexation will likely result in multiple products, as

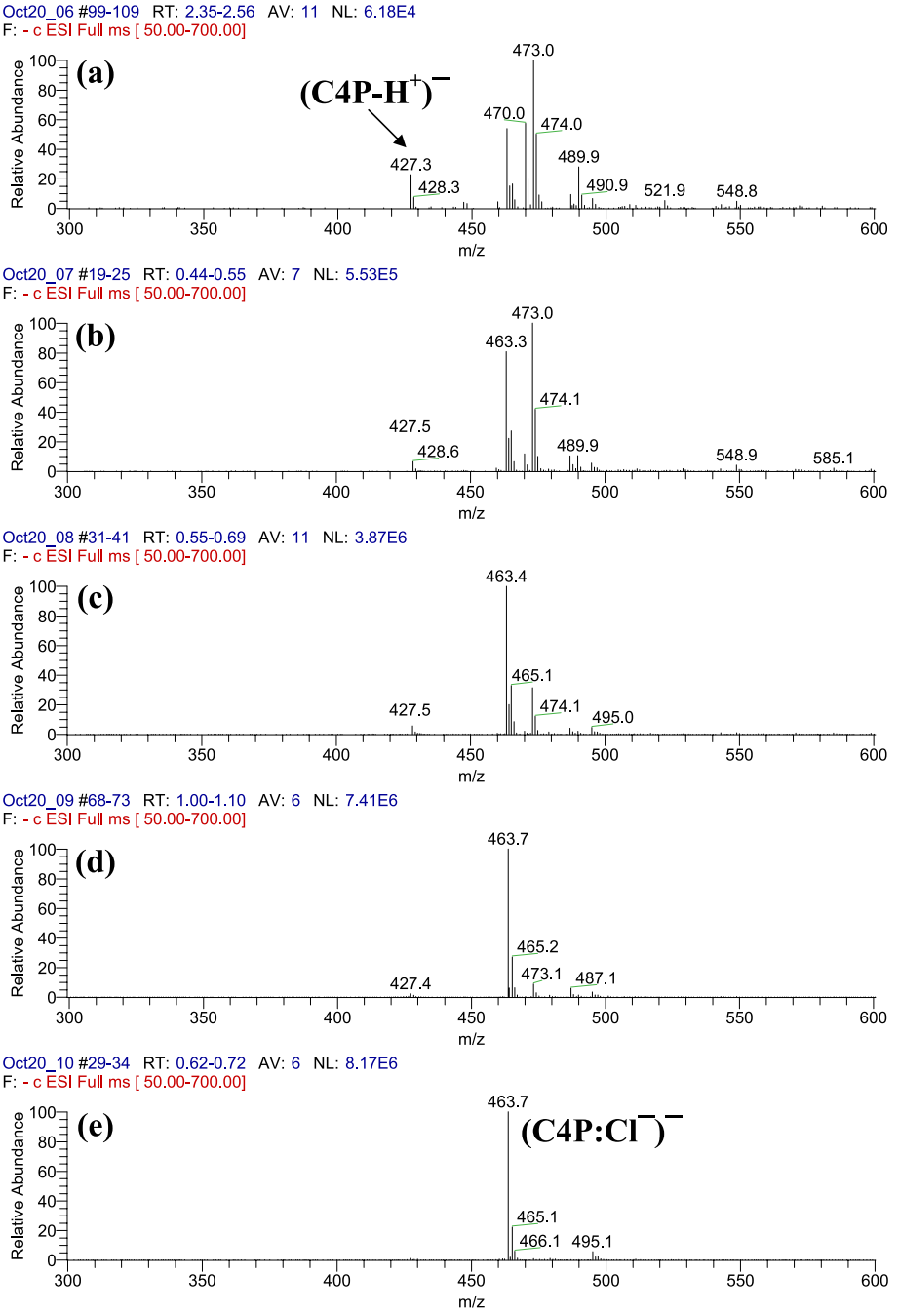


FIG. 8—*Negative electrospray ionization mass spectra of solutions containing |*bital*|meso|*eital*|-octamethylcalix(4)pyrrole and cocaine HCl at (a) 0.10 μg/mL, (b) 1.0 μg/mL, (c) 5.4 μg/mL, (d) 10.8 μg/mL, and (e) 54.4 μg/mL.*

previously illustrated in Fig. 5. Under the current experimental conditions, complexation with C4P is observed for sample concentration levels as low as 0.10 μg/mL for cocaine HCl and dextromethorphan HBr solutions. This corresponds to *c.* 1 ng of sample consumed during each analysis. It must be noted that at these low anion levels, the interference of other anionic species will be significant. Figure 8 shows data for the anion analysis of cocaine HCl solutions as a function of concentration. At high concentrations, the spectra are dominated by the $(C4P:Cl^-)^-$ signal at m/*z* 463. However, as the concentration of chloride in solution is decreased, the spectra are characterized by the presence of the formate complex (m/*z* 473) and by the appearance of the deprotonated C4P species (m/*z* 427).

When multiple salt forms are encountered, reactions with C4P will most likely result in the formation of more than one type of complex and as previously mentioned, the intensity of such complexes in the ESI–MS spectra will not be a measure of the anion concentration, but rather indicative of the affinity with the C4P molecule. Furthermore, under these circumstances, detection of a low-level anion might not be possible in the presence of a higher-concentration, higher-affinity species.

## Conclusions

The cyclic anion-binding agent *meso*-octamethylcalix(4)pyrrole has been used during the identification of various drug salt forms.

The simple addition of this compound to acetonitrile solutions containing chloride, bromide, iodide, nitrate, and acetate salts results in the formation of host–guest complexes that can be readily analyzed using negative mode electrospray ionization–mass spectrometry. For the halide anions chloride and bromide, the characteristic isotopic distribution of the electrospray-generated molecular ions provides further evidence of complex formation and anion identification. For sulfate and phosphate salts, no complex formation is observed. For these larger and higher charged anions, complexation by *meso*-octamethylcalix(4)pyrrole is most likely hindered by steric effects and by the high energy necessary to desolvate the ions prior to the sequestration event.

*Acknowledgments*

The authors would like to thank DEA Research Forensic Chemist Harry F. Skinner for synthesis of some of the ephedrine salts used in this study. The authors also gratefully acknowledge Associate Professor Seth M. Cohen (Department of Chemistry and Biochemistry, University of California, San Diego) for useful discussions. The authors also thank David A. Perez (DEA Laboratory's Secretary) and Esther W. Chege (DEA Supervisory Chemist) for review of the manuscript.

## References

1. 2008 Federal Sentencing Guidelines Manual. Chapter 2—Part D—Offenses Involving Drugs and Narco-Terrorism. Available at: http://www.ussc.gov/2008guid/Chap2_D.htm (accessed January 26, 2009).
2. Rodriguez-Cruz SE. Rapid screening of seized drug exhibits using desorption electrospray ionization mass spectrometry (DESI-MS). Microgram J 2008;6(1–2):10–25.
3. Skoog DA, West DM, Holler FJ. Fundamentals of analytical chemistry. 5th edn. New York, NY: Saunders College Publishing, 1988;75–6, 758–9.
4. McKibben T, Chappell J, Mauslof N, Evans HK. Analyses of inorganic components found in clandestine drug laboratory evidence. J Clandestine Lab Invest Chem Assoc 1995;5(4):19–33.
5. Oulton SR, Skinner HF. Identification of common inorganic acids encountered at clandestine laboratories. J Clandestine Lab Invest Chem Assoc 1998;8(4):17–25.
6. Skinner HF, Oulton SR. Identification and quantitation of hydriodic acid manufactured from iodine, red phosphorus and water. Microgram 1995;28(11):349–57. [Note: This publication is restricted to forensic scientists serving law enforcement agencies.]
7. Haddad PR. Comparison of ion chromatography and capillary electrophoresis for the determination of inorganic ions. J Chromatogr A 1997;770(1-2): 281–90.
8. Lurie IS. The analysis of cations and anions in illicit heroin using capillary electrophoresis with indirect UV detection. J Capillary Electrophor 1996;3(5):237–42.
9. Soga T, Ross GA. Simultaneous detection of inorganic anions, organic acids and metal cations by capillary electrophoresis. J Chromatogr A 1999;834(1-2):65–71.
10. Kage S, Nagata T, Kudo K. Determination of cyanide and thiocyanate in blood by gas chromatography and gas chromatography-mass spectrometry. J Chromatogr B 1996;675(1):27–32.
11. Sakayanagi M, Yamada Y, Sakabe C, Watanabe K, Harigaya Y. Identification of inorganic anions by gas chromatography/mass spectrometry. Forensic Sci Int 2006;157(2-3):134–43.
12. Baeyer A. Berichte der Deutschen Chemischen Gesellschaft. 1886;19:2184–5.
13. Gale PA, Sessler JL, Kral V, Lynch V. Calix[4]pyrrole: old yet new anion-binding agents. J Am Chem Soc 1996;118:5140–1.
14. Gale PA, Sessler JL, Kral V. Calixpyrroles. Chem Commun 1998;1–8.
15. Cafeo G, Kohnke FH, Parisi MF, Nascone RP, La Torre GL, Williams DJ. The elusive $\beta$-unsubstituted calyx[5]pyrrole finally captured. Org Lett 2002;4(16):2695–7.
16. Turner B, Botoshansky M, Eichen Y. Extended calixpyrroles: *meso*-substituted calix[6]pyrroles. Angew Chem Int Edit 1998;37(18): 2475–8.
17. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. Electrospray ionization for mass spectrometry of large biomolecules. Science 1989;246:64–71.
18. Kebarle P, Tang L. From ions in solution to ions in the gas phase, the mechanism of electrospray mass spectrometry. Anal Chem 1993;65:927A–86A.

Additional information and reprint requests:
Sandra E. Rodriguez-Cruz, Ph.D.
U.S. Drug Enforcement Administration
Southwest Laboratory
2815 Scott Street
Vista, CA 92081
E-mail: sandra.e.rodriguez-cruz@usdoj.gov