Annual Journal 2018 - Volume 26

Bangladeshi-American Pharmacists' Association

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ISOLATION AND PURIFICATION OF NATURAL AND SYNTHETIC COMPOUNDS BY CENTRIFUGAL PREPARATIVE CHROMATOGRAPHY

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BAPA Journal is published by Bangladeshi-American Pharmacists' Association

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We deeply regret and mourn the departure of our fellow friends. We miss them a lot and remember them in our prayers. In this day of the Convention we will miss their presence.

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Disclaimer: If we missed anybody's name it is an unintentional mistake.



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26th Annual Convention Programs

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BAPA CONVENTION SCHEDULE AT-A-GLANCE

Day 1	Friday, September 21, 2018
12:00 PM - 8:00 PM Hotel Lobby	Registration
6:00 PM - 7:00 PM	Continuing Education: Immunotherapy in Non- Small Lung Cancer overview Presenter: Dr. Shadman Islam, PharmD, Manager of Scientific Content and Publications, I-O Lung at Bristol Myers Squibb
8:00 PM - 10:00 PM Bayview Ballroom	Dinner and Cultural program
10:00 PM - 12:00 AM	Cultural Show
Day 2	Saturday, September 22, 2018
7:00 AM - 9:00 AM Main Dining Room	Breakfast
9:00 AM - 11:00 AM	Continuing Education: Clinical Drug Development – A Regulatory Overview <i>Presenter:</i> Dr. Naushad Islam, PharmD, Senior Director and Global Regulatory Leader at Johnson &Johnson Pharmaceuticals
9:00 AM - 11:00 AM 11:00 AM - 12:00 PM	Presenter: Dr. Naushad Islam, PharmD, Senior Director and Global



2:00 PM - 5:00 PM	Continuing Education: Medication Error Presenter: Dr. Maria M. Claudio- Saez
4:00 PM - 4:15 PM	Coffee Break and Snacks
7:30 PM - 12:00 AM	Dinner and Cultural Program <i>Keynote Speaker:</i> Stephen Giroux, RPh, Past President of Pharmacists So- ciety of the State of New York (PSSNY) and Past President of the National Community Pharmacists Association (NCPA)
Day 3	Sunday, September 23, 2018
8:00 AM - 10:00 AM Main Dining Room	Breakfast
	Breakfast Continuing Education: Medication Adherence Updates Presenter: Dr. Suhayr Islam, Pharmacy Manager at Health First
Main Dining Room	Continuing Education: Medication Adherence Updates

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EDITORIAL

Message from the **PRESIDENT**



Mohammed SHABBIR TAHER

Dear members of the Bangladeshi-American Pharmacists Association, guests, friends and family, Assalamu alaykum, peace be upon you. We are truly honored to have your presence at our 25th Annual convention. And it is my ultimate honor to serve as your president on our 25th Anniversary.

First I wish to acknowledge the memory of Dr. Abdul Jabbar, the founder of the pharmacy profession in Bangladesh. He was a great teacher, mentor and a father figure to his students. He inspired everyone whose life he touched as he helped them to accomplish great things in life

I graduated in 2009 and soon joined BAPA. It made sense that my involvement in the profession should start in my own backyard with my own community. I served first as secretary, then as a board member, and most recently, as vice president. Over the years, I realized that BAPA isn't just a pharmacy association, it is truly family.

I am proud of the way we always stick by each other. When one of our members has a great accomplishment, we all celebrate together. When there is tragedy, we all suffer the pain together and move forward together. I want to thank all our past presidents for creating such an organization with a family-friendly atmosphere while maintaining its professionalism. Each of you is a great individual who has achieved outstanding professional and social accomplishments. In the end, our past presidents represent an elite group, and for me, it is the highest honor to be counted among them.

May God bless this organization; I hope you all enjoy your time here at Honor's Haven and Resort.

Thank you. **Mohammed Shabbir Taher**, R.Ph., Pharm.D President, BAPA



Message from the VICE PRESIDENT



Fahim AHMAD

Welcome to our annual convention. We celebrate another year of the Bangladeshi-American community moving forward in the profession of pharmacy. I am pleased to welcome all of our vendors that join us every year and hope our relationships will continue to benefit each other. I would like to thank our biggest sponsor, Kinray, for making this convention possible year after year.

This year marks a few changes for our organization. We have increased our use of technology to communicate with our members and new methods to track registration and collect payment. We hope in crease the use of technology as time goes on to expand our ability to connect with you, the members.

This year is special as well because our president, Dr. Mohammed Taher, has been elected to the board of the PSSNY - a great honor for not only him but our entire organization as well.

This organization can only continue with your support and participation. We are always open to any feedback, so please do not hesitate to communicate any questions, comments, or concerns.

We hope you enjoy this years activities and education.

Fahim Ahmad Vice President, BAPA



EDITORIAL

Message from the GENERAL SECRETARY



Nishad HOQUE Thank you for giving me the opportunity to serve as BAPA Secretary. It is the time of the year when all BAPA members meet and greet take place with pleasant and wonderful atmosphere everywhere. I along with the President and Vice President welcome all our fellow members of BAPA in this 2016 convention.

I hope to maintain the level of excellence of my earlier successor and continue creating new path as we move forwards the future.

This is an exciting time for BAPA as the convention is always a wonderful opportunity to meet new people in different areas of pharmacy grounds like industry, hospital, health care and retail. Eating all halal food, enjoying the cultural show and earning Continuing education credits and relaxing for three days from our busy schedules.

I look forward to welcome all new comer and new pharmacists of 2015 and 2016 and their family members. At the end best of luck for this year convention and current committee who is working hard day and night to make this even successful.

Thank you

Nishad Hoque , Pharm D, R.Ph Secretary, BAPA



EDITORIAL

Message from the TREASURER



Fariha

I am honored to be part of the Executive Board and to serve as the Treasurer of BAPA for the first time. This organization has been a integral part of my family life since its inception and I would like to thank the entire BAPA community for being a positive influence in my upbringing. As the daughter of the late past President, Abu M. Kabir, I take great pride in expanding his legacy and devotion to BAPA. I have always been impressed with the numerous accomplishments of our organization and our dedicated leadership among the pharmacy community around the world over the years. I am particularly motivated to work on supporting our efforts for continued funding of student scholarships and awards as well as providing funds for further research on groups.

As an active practicing pharmacist for four years, I have thoroughly enjoyed the opportunity to join with colleagues who have become family in efforts and activities to spread awareness of BAPA's mission. I am excited to be directly involved in the development of our organization and am positive that the future of BAPA will only flourish. As treasurer, I have worked in collaboration to recruit the largest number of new members in BAPA history and hope to grow the organization further. My goal is to work closely with the new generation of pharmacists to raise funds and establish relations with other healthcare professionals to bring BAPA to the forefront of professional pharmacist organizations.

On behalf of the rest of the Executive Board, I would like to show our appreciation to our President and Vice President for continuously working to evolve BAPA into a stronger organization by bringing innovative ideas while maintaining BAPA's core values.

Fariha Kabir, Pharm.D, R.Ph Treasurer, BAPA







Articles

Isolation and Purification of Natural and Synthetic Compounds by Centrifugal Preparative Chromatography

Abstract. Liquid chromatography, generally, relates to the separation of a single component from a multicomponent mixture by moving components through an adsorbent, which interacts with liquid mobile phase, by means of capillary (i.e., thin-layer chromatography), gravity (column chromatography), pressure (high pressure liquid chromatography), and centrifugal forces generated by high speed rotation (centrifugal planar chromatography). In the area of centrifugal preparative chromatography, we herein report method of preparation of rotors with regular and reversed phase silica gel sorbent layers. The rotors consist of sorbent layers packed between two circular glass discs (sandwich type) and can be used in an appropriate instrument for centrifugal chromatography, such as using our prototype unit UM8390, as well as commercially available Chromatotron[®] and CycloGraph[®]. Mixtures of organic compounds, polar and /or semi-polar, including plant extracts and drug mixtures with close R_f values (or R_t; retention time) can be separated and /or purified in a preparative and / or semi-preparative scale using the normal or RP rotors, eluting with mixtures of aqueous-based or organic solvents. We also herein report few application notes, using normal or RP Chromatorotors and commercial plates, for the separation of various compounds, including the diastereoisomeric alkaloids banistenosides A and B from Banisteriopsis caapi, the antimalarial drug artemisinin and artemisinic acid from Artemisia annua, and isomeric turmerone mixture from Turmeric, as well as enantiomeric synthetic antimalarial drugs (+) and (-)- primaquine phathalate using a chiral rotor.

Introduction. Liquid chromatography is a technique used to separate a mixture of compounds into its individual components. The separation occurs based on the interactions of the compound mixture with the stationary mobile phases. When separating a mixture, many mobile/ stationary phase combinations can be employed, with several different types of chromatographic techniques that are distinguished on the basis of physical states of phases. The most popular chromatography technique is solid-liquid chromatography, features a mobile phase (liquid), which slowly sieves down through the stationary phase (solid), eluting the separated components. The interaction with the liquid media define the nature of the chromatography, such as for capillary (thin-layer chromatography (TLC)], gravity [column chromatography (CC)], pressure [high pressure liquid chromatography (HPLC)] or centrifugal partition chromatography]. Centrifugal preparative chromatography (CPC) is a convenient, reliable and economic method for preparative scale separation of organic compound mixture, including natural products.^{1,2} CPC devices have long been recorded in the art of chromatographic separation. The discovery of axial flow chromatography



long been recorded in the art of chromatographic separation. The discovery of axial flow chromatography by Hopf in the late 1940s led to the emergence of radial chromatography,³ known as centrifugal layer chromatography. This was the very first device, named "Chromatofuge", for a method adopted to separate compounds using centrifugal forces.³ Later, it was modified for adsorption chromatography^{4,5} and then developed further in the 1980s by Nyiredy and renamed as rotation planar chromatography (RPC).⁶ This was catered for micro-preparative, preparative and analytical work. CPC is currently applied to preparative separation using commercially available instruments, Chromatotron[®],⁷ Cyclograph[®],⁸ and Rotachrom P,⁹ while another RPC instrument ExtraChrom,¹⁰ used for preparative work is not available commercially.

In general, the method of centrifugal chromatography is suitable for separating both natural and synthetic compounds. Chromatotron[®] and Cyclograph[®], the two CPC instruments are operated by the same principal involving movement of the mobile phase by centrifugal forces through a thin layer of sorbent coated on either a circular glass or plastic plate with the aid of a binder. Centrifugal forces are generated by the planar circular motion or rotation of the plate mounted on the inner chamber of the vessel of the instrument. Both instruments are designed for preparative and semi-preparative scale separation of compounds *either* with silica gel or alumina sorbents (see examples 1 and 2). The use of only silica gel or alumina as sorbents severely limits the nature of compounds separable by these discs, as well as the composition of the solvent used for the separation. It is well-known that polar compounds like glycosides, anthocyanidins, polyphenols, quaternary alkaloids, and sugars, are either not separable or only separable with great difficulty and /or with very low yields using either silica gel or alumina as sorbents. The limitation in the use of water or acidic solvents is due to the fact that gypsum (CaSO₄), used as a binder for silica gel and alumina rotors, is sparingly soluble in water or acid, and eventually washed out slowly from the rotors during elution, thus destroying the rotors. In addition, many non-polar or mid-polar compounds with close R_f values in silica gel or alumina (TLC) sorbents demonstrate improved separation in RP sorbent, and, therefore, RP Chromatorotors would also be useful for the separation of these compounds.

We herein report the preparation of a separating device containing a layer of binder free sorbents (i.e., C18 silica gel/ regular silica gel/ alumina) packed between the two circular rotors for CPC separation.^{11,12} These sorbent layers contained fluorescent indicators, which allow the visualization of the separation process under UV lights. In addition, we also report application of the normal silica gel, alumina and RP rotor used in the separation of various non-polar to polar compounds. As for example, the two polar diastereoisomeric alkaloids banistenosides A and B from *Banisteriopsis caapi*, the antimalarial small molecules artemisinin and artemisinic acid from *Artemisia annua*, and isomeric α - and β -turmerone



mixtures from Turmeric, using RP rotors, together with the separation of enantiomeric synthetic antimalarial drugs (+) and (-)- primaquine phathalate using a chiral rotor.

Discussion. The development of Chromatorotor technology^{11,12} (UM8270) was based on the principles and procedures of currently available devices for CPC, so that the rotor can be used in these instruments for normal separation work for silica gel and alumina sorbents using non-aqeuous organic solvents. In addition, we have devised an improvement of the existing instrument (Chromatotron), named UM8390 to carry out the separation works.

Preparation of the rotor using a binder free C18 SiO₂ sorbent layer enabled RP mode of separation with water-based solvents, which was not executed fully for separation work by commercial devices. A detailed experimental procedures including the preparation of custom rotors has been published previously.^{11,12} The RP rotors are conveniently prepared *in-situ*, using our technology UM8390 Three different rotors with 1, 3, and 6 mm thickness can be prepared depending on the sample load. Rotors can be regenerated by washing with appropriate solvents at high speed rotation after separation. However, the empty rotor is not restricted to RP sorbent only and can be filled with regular silica gel (SiO₂) for normal phase separation, silica gel modified with functional groups and/or chiral groups, alumina, polyamide or sephadex resins and used for regular phase of chromatographic separation.



Figure 1. (A) Planar sectional view of the rotor (in mm); (B) empty and (C) RP silica gel filled 3 mm rotor; (D) sorbent filled rotor in a Chromatotron instrument.

The standard CPC separation of lignans and alkaloids using commercial silica gel coated rotor (example 1) and in-house alumina coated rotor (example 2), respectively, were carried out (vide infra). Application of the RP rotor and its usefulness is discussed in the following application notes for the separation of different classes of compounds from natural products, including polar alkaloids, sesquiterpenes, monoterpenes and synthetic racemic alkaloids, using H₂O based solvents, such as H₂O-Me₂CO, H₂O-MeOH and H₂O-MeCN.



Example 1. Separation of Lignans from Magnolia grandiflora

The extracts of magnolia have diverse traditional medicinal uses and recently the lignans isolated from the seed showed cannabinoid receptor CB-2 inhibitory activities. Three small molecules lignans were isolated from Magonolia seeds using a regular 6 mm silica gel P₂₅₄ commercial rotor. 4-*O*-methylhonokiol, grandifloralignan and magnolol were isolated (Fig. 2). The same experiment was conducted with Chromatorotor packed with flash silica gel and eluted with the same solvent (EtOAc : Hex) afforded the same result.

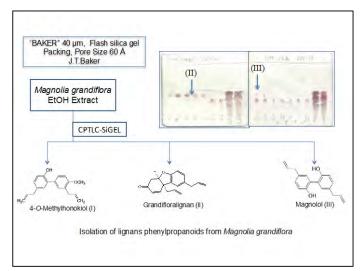


Figure 2. Structures and TLC spots for the separation of lignans from Magnolia seeds

Example 2. Separation of benzylisoquinoline alkaloids from Nelumbo nucifera

N. nucifera (Indian lotus) is an aquatic plant with enormous medicinal properties. Several benzyl isoquinoline alkaloids were isolated from lotus flower using an alumina rotor. Due to unavailability of alumina plate from commercial sources, a 4 mm neutral Al₂O₃ rotor was prepared and 1 g of basic alkaloidal fraction was applied to the rotor, and eluted with DCM: MeOH: NH₃. Two aporphines (nuciferine and nor-nuciferine) and three benzyltetrahydroisoquinolines (coclaurine, *N*-methyl- and *O*-methyl-coclaurines) were isolated in pure forms (Fig. 3).¹³



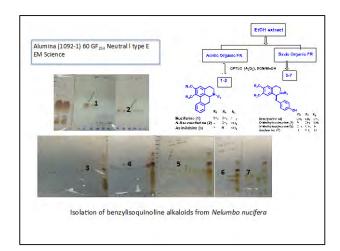
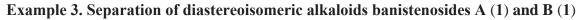


Figure 3. Structures and TLC spots for the separation of benzylisoquinoline alkaloids from Lotus



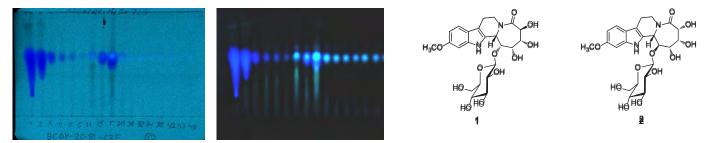
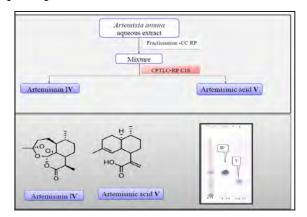


Figure 4. Images of TLC spots (under short and long UV lights) for the separation of banistenoside A (1) and B (2)

Banisteriopsis caapi is a South American plant used for the preparation of a sacramental drink called Ayahausca. In the first example of RP-Chromatorotor drived separation work, two diastereoisomeric alkaloids banistenosides A (1) and B (2), with almost identical R_f values on RP TLC (R_f 0.57 and 0.58, C18 silica gel, solvent: H₂O-Me₂CO, 3:7), could only be separated by repeated RP column chromatography with great difficulty, and successfully resolved as only acetate derivatives after acetylation of a mixture of 1 and 2.¹⁴ Using a 3 mm C18 silica gel rotor, the two polar alkaloids, 1 and 2, were separated with >95% purity (by NMR). The compounds were observed as two distinct fluorescent bands under short and long wave UV light, with the RP rotor (Fig. 4). They were identified by comparison of spectroscopic data (NMR and ESIMS) of their respective heptaacetates with published data.¹⁴



Example 4. Separation of sesquiterpenes and artemisinin from artemisinic acid

Figure 5. Structures and TLC spots for the separation of artemisinin and artemisinic acid

The separation of artemisinin, an antimalarial drug with sesquiterpene endoperoxide moeity, from its precursor artemisinic acid was always challenging since they tend to co-elute from silica gel column chromatography. Following a report of the isolation of artemisinin and artemisinic acid from *A. annua* using preparative RP-HPLC, we now have separated these two sesquiterpenes from the *n*-hexane extract using a RP rotor, eluted with MeCN-H₂O (R_f 4.2 and 2.1 respectively, C18 silica gel, solvent: H₂O-Me₂CO 4:6) (Fig. 5), in good yields (i.e., 1.1% and 2.7% of *n*-hexane extract, respectively). The spectroscopic data for the separated compounds, artemisinin, and artemisinic acid were in agreement with those reported previously.¹⁵

Example 5. The isolation of turmerones from turmeric

We examined the EtOH extract of turmeric, which was rich in curcuminoids, together with the small molecules turmerones, which has anticancer and anti-inflammatory effects. Crude turmeric EtOH extract was fractionated by Si gel cartridge using Hex-EtOAc (75:25) as solvent, afforded turmerone enriched fraction, a mixture of three isomeric compounds, α -, β - and Ar-turmerone. 1 mm disc has been prepared using 30 g C18 spherical silica gel, porosity 70 Å, and particle Size 20-45 μ M. 80 mg mixture sample was loaded on the disc using 40% MeCN in H₂O as an eluent. This separation has been planned to test the efficiency of quick separation using our system, where only 6 fractions have been collected and the separation has been executed in an hour. The target compound was the middle spot in RP-TLC (see figure below) and the mixture was subsequently separated to give α - and β -turmerone (R_f 0.27 and 0.15, C18 silica gel, solvent: H₂O-MeCN, 3:7) in good yields (Fig. 6). The recovery was \geq 95% and two fractions have been shown to contain turemerone in higher purity (\geq 90%) *via* TLC and RP18 TLC, using Hex-EtOAc (75:25)



and MeCN-H₂O (85:15) as eluting solvent, respectively. This separation provided a model of high resolution, and low solvent consumption that highlights the green chemistry of the developed system.

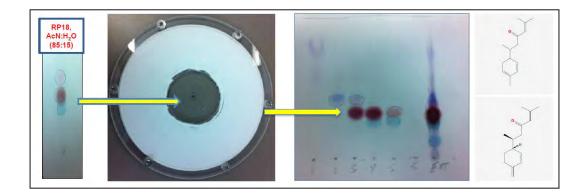


Figure 6. Images of TLC spots and rotor used for the separation of α -turmerone and β -turmerone

Example 6. Preparation of chiral rotor and separation of racemic mixtures

This separation has been planned to test the wide applicability of the developed system using the most challenge chiral separation. The enantiomeric mixture of the antimalarial compound primaquine phthalate was separated using a chiral rotor (ChiralPak OD, 20 μ M Bulk Material; 14020 Unit: G; Interchim Inc.). 1 mm chiral rotor was prepared using 30 g chiral sorbent and 100 mg of synthetic racemic mixture was applied to the chiral rotor for separation. A gradient elution was applied using water-MeCN (80%-20% water in MeCN) and sixty tubes, each 10 ml, were collected. Fractions were pooled based on eluting solvent and (F-25-30 eluted with water-MeCN, 80:20) have been combined and dried where the specific rotation was -120. Tubes # 55-60 eluted with water-MeCN (4:96) have been combined and dried where the Specific Rotation was +80. This suggests that the (+)- enantiomer was eluted first, followed by (-)- enantiomer. A chiral HPLC¹⁶ (Fig. 7) of the fraction showed clear separation of the enantiomers in higher purity (\geq 90%) of each isomer.

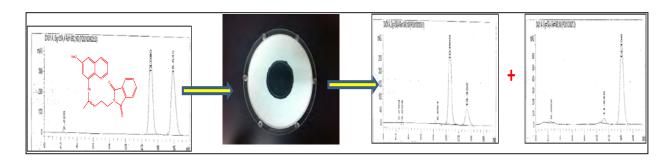




Figure 7. Images of chiral HPLC peaks of (+)- and (-)-primaquine phthalate before and after separation from chiral chromatorotor

The results confirmed the wide applicability and the use of fine powered sorbent for the separation of this Chromatorotor system that extended to the chiral field.

Conclusion. In conclusion, the RP Chromatorotors have demonstrated the ability to separate mixtures of polar diastereoisomeric alkaloids with almost identical R_f values, as shown in the separation of banistenosides A and B. In addition, two semi-polar sesquiterpenes artemisinin and artemisinic acid, and monoterpenes α - and β - turmerones were convincingly separated by RP rotor. Furthermore, this technology can also be used for the separation chiral compounds (as evidence by the separation of racemic primaquine phthalate) by chiral rotors. Compared to preparative HPLC, this technology has advantages of being economical both in terms of instrumentation and accessories and it also overcomes the loading capacity and limitations encountered in preparative HPLC columns. Compared with normal RP column chromatography, it has the advantage of being both fast and efficient in resolution of mixtures visualized under UV fluorescence, thereby separation of compounds can be observed in real time as bands. Finally, this technique is also environmentally friendly as it consumes less and inexpensive solvents, and low usage of sorbent since rotors are reusable.

Acknowledgement. The authors sincerely thank Drs. Larry Walker and Walt Chambliss, Ms. Allyson Best for support and advice for the project, Dr. Francis Machumo for technical assistance, and Dr. Dhammika Nanayakkara for providing racemic primaquine phthalate sample and Dr. Ahmed Galal for reference standards. This work was supported the by National Center Natural Product Research, University of Mississippi.

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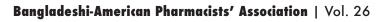






















































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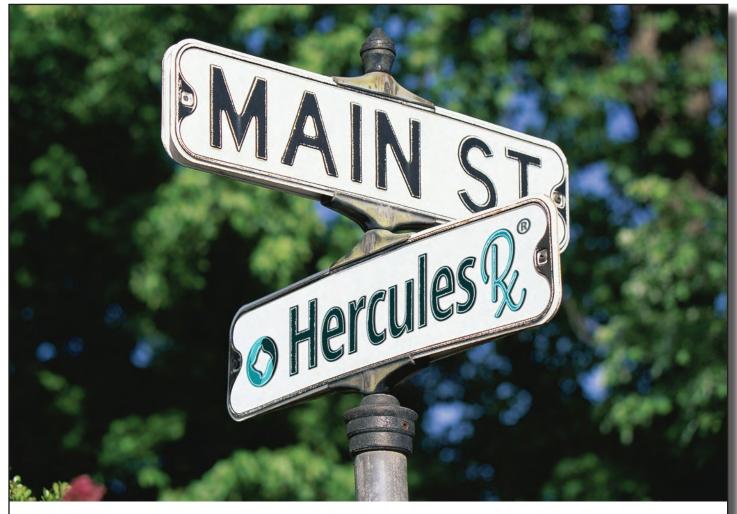
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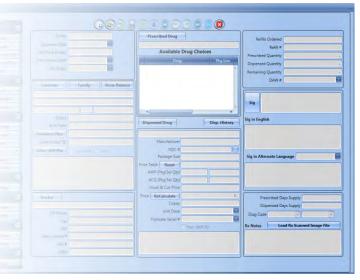
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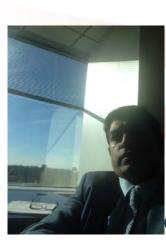
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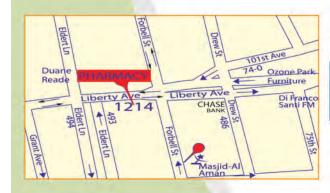
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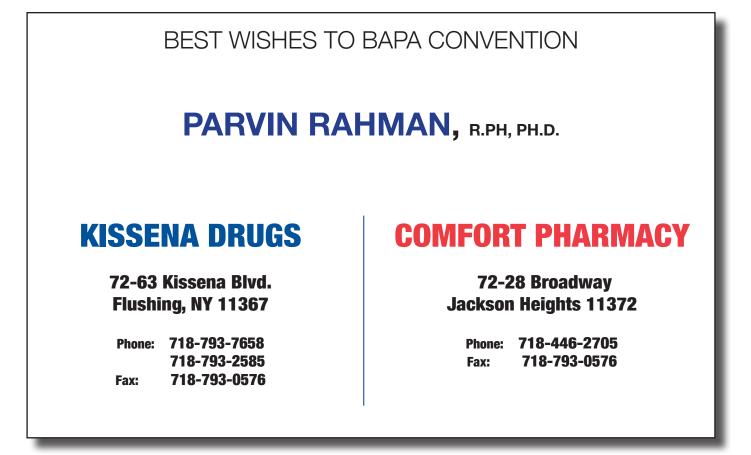
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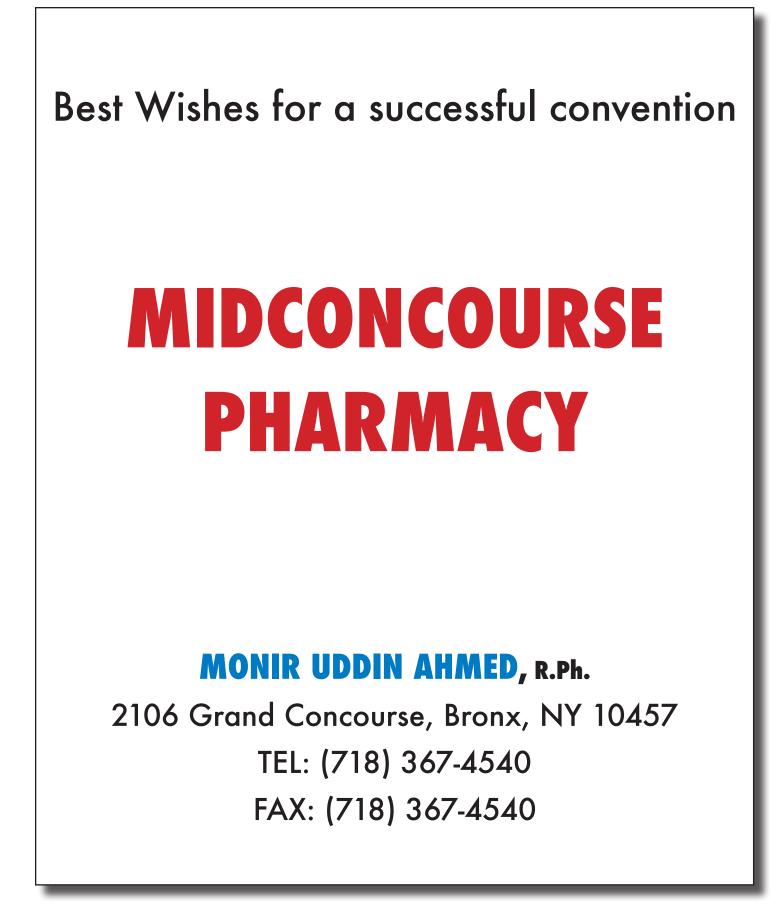
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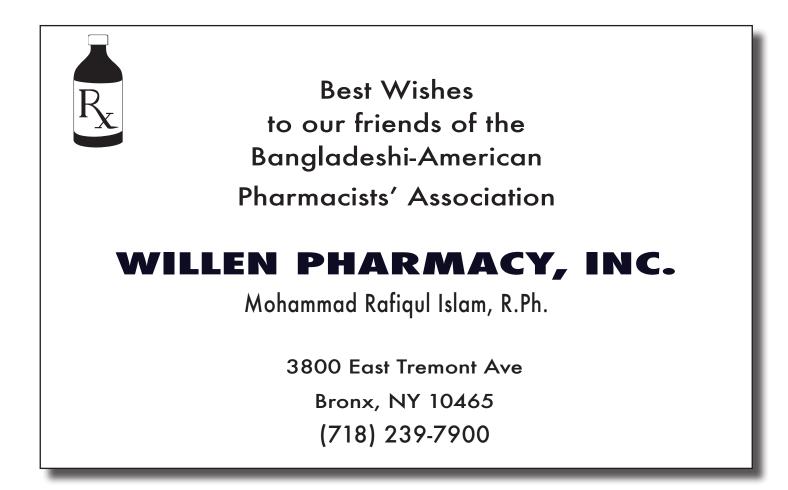
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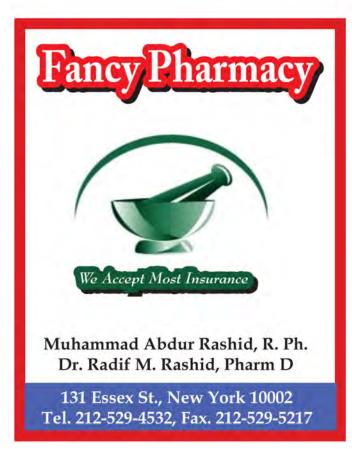
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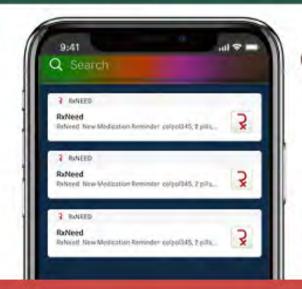
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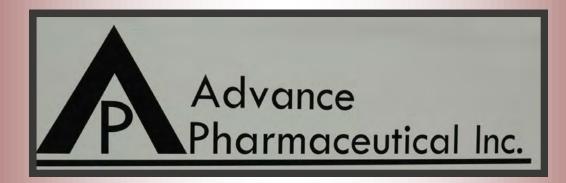


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